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Drinking Water Criteria Document for
Heptachlor, Heptachlor Epoxide and
Chlordane (Final Draft)

(U.S.) Environmental Protection Agency
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DRINKING WATER CRITERIA DOCUMENT FOR
HEPTACHLOR, HEPTACHLOR EPOXIDE AND
CHLORDANE

Prepared for

OFFICE OF DRINKING WATER

Prepared by

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LIST OF ABBREVIATIONS

ADI	Acceptable daily intake
AEL	Adverse effect level
CCl ₄	Carbon tetrachloride
CNS	Central nervous system
FEL	Frank effect level
GC-MS	Gas chromatography-mass spectrometry
GI	Gastrointestinal
GLC	Gas liquid chromatography
GOT	Glutamine oxalacetic transaminase
GPT	Glutamic pyruvic transaminase
HA	Health advisory
LOAEL	Lowest-observed-adverse-effect level
MW	Molecular weight
NOAEL	No-observed-adverse-effect level
NOEL	No-observed-effect level
ppm	Parts per million
TLC	Thin-layer chromatography
TWA	Time-weighted average

I. SUMMARY

Chlordane (MW 409.76) and heptachlor (MW 373.32) are structurally related chlorinated hydrocarbon insecticides used to control termites and pests on field crops. Heptachlor epoxide (MW 389.32) is a degradation product of heptachlor. Technical grade chlordane consists of approximately equal parts cis- and trans- isomers, along with heptachlor and other impurities, and has a low water solubility. Technical grade heptachlor consists of ~73% heptachlor and 22% trans-chlordane, and also has a low water solubility. Heptachlor epoxide is slightly water soluble.

Neither chlordane nor heptachlor epoxide undergoes significant chemical reactions in drinking water; however, both may volatilize during aeration of drinking water. Chlordane, heptachlor and heptachlor epoxide may sorb to organic particulate matter and be removed from drinking water during filtration. Heptachlor may hydrolyze in drinking water and may undergo oxidation with singlet oxygen during the water treatment process. Heptachlor will probably volatilize faster than chlordane and heptachlor epoxide during aeration.

The absorption of chlordane by the gastrointestinal tract of animals can be inferred from data on its systemic toxicity and from excretion data. Rats absorbed at least 2-8%, while rabbits absorbed at least 30% of orally administered chlordane. Humans also absorbed chlordane after accidental poisoning. Chlordane and/or metabolites are rapidly distributed to tissues of animals, with highest levels detected in adipose tissue where it persists as the metabolite oxychlordane. Oxychlordane has been detected in human

adipose tissue. At least 13 or 14 fecal metabolites and a urinary glucuronide conjugate have been isolated in rats. Metabolism involves dehydrogenation, epoxidation (to oxychlordanes), hydroxylation and dechlorination reactions. Metabolism by human microsomes in vitro is similar to metabolism by rat microsomes in vitro. The major route of excretion of chlordanes and metabolites in rats is via the feces with 50% of a single oral dose excreted in 10 days. Rabbits eliminate more chlordanes metabolites in the urine than in the feces. In humans, the half-time for elimination from serum has been measured at 21-88 days. Oxychlordanes has been found in the milk of lactating women.

Heptachlor is absorbed from the gastrointestinal tract of rats; residues appear in blood within an hour of oral dosing. Absorption of heptachlor by humans can be inferred from reports of heptachlor epoxide levels in adipose tissue of the general population. Heptachlor epoxide, the major metabolite of heptachlor, is distributed to tissues of animals; the highest levels are detected in adipose tissue where it persists. Heptachlor is epoxidized to heptachlor epoxide that is dechlorinated and hydroxylated to a metabolite eliminated in the feces. Other routes of metabolism involve epoxidation of other intermediates. Rat liver microsomes form 4 times more heptachlor epoxide than human microsomes; otherwise, in vitro metabolism is similar. Rats eliminate >50% of administered heptachlor as fecal metabolites; <5% is eliminated in urine.

The acute oral LD₅₀ of chlordanes (in a lipophilic vehicle) is ~350 mg/kg bw in rats, 390 mg/kg bw in mice and 1720 mg/kg bw in hamsters. Neonatal rats are less sensitive to chlordanes toxicity than are adults.

Symptoms of acute intoxication include CNS disturbances, such as irritability, tremors and convulsions. Sublethal doses result in microsomal enzyme induction and enhanced gluconeogenesis. Subchronic dietary administration of chlordane to rats and mice has resulted in changes in prostate homeostasis and microsomal enzyme induction. The no-effect level for enzyme induction is <5 mg/kg diet. In chronic dietary studies, a no-effect level of chlordane in rats for histopathologic changes in liver, kidney, lungs, pancreas, stomach, adrenals, thyroid, thymus, lymph, testes, ovaries, heart and spleen; for food consumption; for growth rate; and for mortality was 5 mg/kg diet. At 10 mg/kg diet, mild liver changes (e.g., hepatocytomegaly) were observed. At 150-300 mg/kg, progressively more severe histopathologic damage to liver and other organs occurred.

Chlordane is hepatocarcinogenic in mice, but not in rats. Equivocal results were obtained from mutagenicity assays. Chlordane was not teratogenic or fetotoxic to rats in one study, but pups nursed by treated dams developed symptoms of toxicity. Chlordane administration resulted in reduced fertility in mice.

Heptachlor is more toxic than chlordane to laboratory animals. The acute oral LD₅₀ of heptachlor is ~100 mg/kg bw for rats, 70 mg/kg bw for mice and 105 mg/kg bw for hamsters. Neonatal rats were less sensitive than adult rats to heptachlor administered intraperitoneally. The acute LD₅₀ of heptachlor epoxide in rats is ~60 mg/kg bw. Symptoms of acute intoxication of heptachlor and heptachlor epoxide include tremors, convulsions, paralysis and hypothermia. Sublethal dietary doses resulted in microsomal enzyme induction; the no-effect level was <2 mg/kg diet. Moderate liver

damage in rats was accompanied by increased serum levels of hepatic enzymes at dietary levels of 7 or 12 mg/kg bw/day for 14 days; by 28 days tolerance appeared to develop.

Subchronic dietary exposure to heptachlor has been associated with changes in rat prostate homeostasis and induction of hepatic microsomal enzymes. Chronic dietary exposure to heptachlor or heptachlor epoxide has resulted in hepatocytomegaly, hyperplasia, hepatic vein thrombosis and cirrhosis in mice.

Heptachlor and heptachlor epoxide induced hepatocellular carcinoma in mice. Heptachlor epoxide also induced liver carcinomas in rats. The incidence of neoplastic liver lesions in rats is greater after heptachlor/heptachlor epoxide treatment than after chlordane exposure. The results of mutagenicity tests are equivocal. In one study for which a dose was not clearly defined, heptachlor was reported to cause reduced litter sizes of rats during several generations of dietary exposure.

For humans, clinical case studies of acute exposure to chlordane describe CNS disturbances. An estimated dose of 0.14 mg/kg bw chlordane resulted in convulsions and seizures in a child. Death occurred in a woman who ingested 104 mg chlordane/kg bw. Blood dyscrasias, such as aplastic anemia, leukemia and pancytopenia, have been described in cases involving exposure to chlordane and heptachlor, either alone or in combination with other pesticides. Neuroblastoma has been associated with pre- and/or post-natal exposure to chlordane and heptachlor. Epidemiological studies have linked lung cancer and cerebrovascular disease with chlordane and heptachlor exposure.

Several studies on the mechanism of neurotoxicity indicate that EEG changes, acetylcholine content of brain, ammonia and glutamate content of brain, and inhibition of synaptosomal ATPases are involved in CNS toxicity. Other mechanisms studied included enhanced gluconeogenesis and effects on cellular respiration. Phenobarbital and turpentine enhanced the toxicity of chlordane and heptachlor in rats. Chlordane pretreatment enhanced the toxicity of CCl_4 . The content and quality of protein in the diet affects the toxicity of chlordane and heptachlor. An epigenetic mechanism for carcinogenicity has been suggested.

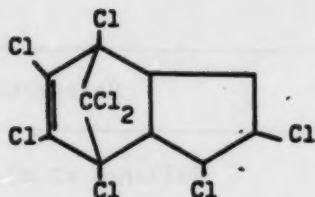
Quantitative risk assessment for chlordane was based on animal data since human data were insufficient. Satisfactory dose-response data was not available for a derivation of a 1-day HA for chlordane. A 10-day HA for chlordane for a 10 kg child is 0.063 mg/l and for a 70 kg adult is 0.22 mg/l. An AADI derived from chlordane toxicity data is 0.03 mg/l or 0.005 mg/l if one assumes only 20% of the acceptable daily intake is from drinking water. The concentrations of chlordane in water corresponding to an increased lifetime risk of cancer of 10^{-4} , 10^{-5} and 10^{-6} are 2.18, 0.218 and 0.0218 $\mu\text{g/l}$, respectively.

Quantitative risk assessment for heptachlor and heptachlor epoxide was based on animal toxicity data, since human data were insufficient. A 1-day HA for heptachlor for a 10 kg child is 10 $\mu\text{g/l}$ and for a 70 kg adult is 35 $\mu\text{g/l}$. Because there was a paucity of data to derive 10-day HAs and because there was some indication that rats develop tolerance to heptachlor, the values for 1-day HAs were recommended to serve as 10-day HAs, as well.

An AADI derived from heptachlor toxicity data is 2.5 $\mu\text{g}/\text{l}$ or 0.5 $\mu\text{g}/\text{l}$ if one assumes only 20% of the acceptable daily intake is from drinking water. An AADI derived from heptachlor epoxide toxicity data is 1.0 $\mu\text{g}/\text{l}$ or 0.2 $\mu\text{g}/\text{l}$ if one assumes only 20% of the acceptable daily intake is from drinking water. The concentrations of heptachlor in drinking water corresponding to increased lifetime risk of cancer of 10^{-4} , 10^{-5} and 10^{-6} are 1.04, 0.104 and 0.0104 $\mu\text{g}/\text{l}$, respectively. The concentrations of heptachlor epoxide in drinking water corresponding to increased lifetime risk of cancer of 10^{-4} , 10^{-5} and 10^{-6} are 60.49, 6.049 and 0.6049 ng/l , respectively. However, it should be noted that the NAS (1977) has taken the position that "Before limits for...heptachlor epoxide in drinking water can be established, more toxicological data must be gathered and evaluated" (NAS, 1977). The IARC (1977) also contends that "There is limited evidence that heptachlor epoxide is carcinogenic in experimental animals."

II. GENERAL INFORMATION AND PROPERTIES

Chlordane



Molecular formula: $C_{10}H_6Cl_8$

Molecular weight: 409.76

RTECS number: PB 98000

Chemical Abstracts Service number: 57-74-9

Chlordane is the common name for 1,2,4,5,6,7,8,8-octachloro-2,3,3a,4,7,7a-hexahydro-4,7-methano-1H-indene. This chemical is also known as dichlorodene; 1,2,4,5,6,7,8,8-octachloro-4,7-methano-3a,4,7,7a-tetrahydroindane; and 1,2,4,5,6,7,8,8-octachloro-3a,4,7,7a-tetrahydro-4,7-methanindan, among others. Some trade names of chlordane include Octachlor, Velsicol 1068, Toxichlor and Dowchlor (IARC, 1979a).

Chlordane is a contact insecticide (BCPC, 1977) and has been used in the control of cutworms, ants, root weevils, rose beetles, grasshoppers, grubs and termites (Deichmann, 1981). Technical grade chlordane consists of 24% transchlordane, 21.5% chlordene isomers, 19% cis-chlordane (CAS No. 5103-74-2; RTECS No. PC01750), 10% heptachlor, 7% nonachlor and 18.5% other miscellaneous impurities (IARC, 1979a).

The chemical and physical properties of chlordane, a viscous amber liquid (IARC, 1979a), are given in Table II-1.

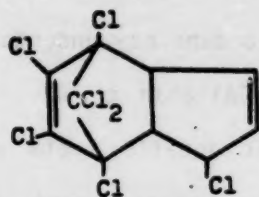
TABLE II-1
Chemical and Physical Properties of Chlordane

Property	Value or Comment	Reference
Boiling point	- 175°C at 2 mm	Callahan et al., 1979
Melting point	106-107°C (cis-isomer) 104-105°C (trans-isomer)	BCPC, 1977
Specific gravity	1.59-1.63 at 25°C	Deichmann, 1981
Vapor pressure	1 x 10 ⁻⁵ mm Hg at 25°C	IARC, 1979a
Solubility	9 µg/l at 25°C in water for technical grade and 56 µg/l in water for cis:trans (75:25) chlordane; miscible with aliphatic and aromatic hydrocarbon solvents	Verschueren, 1983; Deichmann, 1981
Evaporation half-life (t _{1/2}) from water	28 hours (trans-isomer) from 1 meter depth; 33 hours (cis-isomer) from 1 meter depth	Atlas et al., 1982
Log octanol/water partition coefficient	3.32	Rao and Davidson, 1982
Conversion factor	1 mg/l = 59.7 ppm and 1 ppm = 16.76 mg/m ³ at 25°C; 760 mm Hg	Deichmann, 1981

The fate of chlordane in aquatic media cannot be assessed with certainty. Although there is evidence that chlordane may undergo sensitized (acetone, etc.) photolysis in aquatic media (Callahan et al., 1979), the direct photolysis of this compound in ambient aquatic media without any sensitizer appears to be an insignificant process (Verschuere, 1983; Mabey et al., 1981). From the oxidation rate constant values given by Mabey et al. (1981), the two likely oxidation processes in aquatic media, involving singlet oxygen and peroxy/radical, do not appear to be significant processes for chlordane. Similarly, both hydrolysis and biodegradation of this compound in ambient aquatic media are not likely to be significant fate-determining processes (Mabey et al., 1981; Callahan et al., 1979; Tabak et al., 1981). The three likely processes that may determine the fate of this compound in aquatic media are evaporation, sorption and bioaccumulation. The evaporative half-life of this compound from 1 m depth of water was estimated to be 28-33 hours (Atlas et al., 1982). Thus, although the evaporation from aquatic media may not be rapid, it could be significant in the absence of any other faster processes.

From the sediment-water sorption coefficient value (1.4×10^{-3}) given by Mabey et al. (1981), chlordane is expected to be significantly sorbed from the aquatic phase to suspended particles and sediment in water. Therefore, sorption may play a significant role in determining the aquatic fate of chlordane (Callahan et al., 1979). In the treatment of wastewater, a 2.1 mg/l carbon dose was required to reduce the pollutant concentration from 0.1 to 0.01 mg/l (U.S. EPA, 1981).

Heptachlor



Molecular formula: $C_{10}H_5Cl_7$

Molecular weight: 373.32

RTECS number: PC 07000

Chemical Abstracts Service number: 76-44-8

Heptachlor is the common name for 1,4,5,6,7,8,8-heptachlor-3a,4,7,7a-tetrahydro-4,7-methano-1H-indene. This chemical is also known as 3-chloro-chlordene and 3,4,5,6,7,8,8a-heptachlorodicyclopentadiene. Some trade names of heptachlor include Velsicol 104, Drinox, Heptagran, Rhodiachlor and E3314 (IARC, 1979b).

Heptachlor is a contact insecticide with some fumigant action (BCPC, 1977) used to control termites and insects that attack field crops (Deichmann, 1981). Technical heptachlor consists of ~73% heptachlor, 22% trans-chlordane and 5% nonachlor (Deichmann, 1981).

The chemical and physical properties of 99% pure heptachlor (unless otherwise stated), a white crystalline solid (IARC, 1979b), are given in Table II-2.

TABLE II-2

Chemical and Physical Properties of Heptachlor

Property	Value or Description	Reference
Boiling point	135-145°C at 1-1.5 mm Hg	IARC, 1979b
Melting point	93°C	IARC, 1979b
Specific gravity	1.57-1.59 (unknown purity)	Deichmann, 1981
Vapor pressure	3×10^{-4} mm Hg at 25°C	IARC, 1979b
Absorption spectrometry	$\lambda_{\max}$ 236 nm ($E_1^1 = 1611$), 309 nm ($E = 270$), 328 nm ($E_1^1 = 203$ in ethanol)	IARC, 1979b
Log octanol/water partition coefficient	3.87 (measured) 5.44*	Rao and Davidson, 1982 Mackay, 1982
Solubility	in water, 0.056 mg/l at 25°C; soluble in many organic solvents	BCPC, 1977; IARC, 1979b
Conversion factor	1 mg/l = 65.1 ppm and 1 ppm = 15.35 mg/m ³ at 25°C, 760 mm Hg	Deichmann, 1981

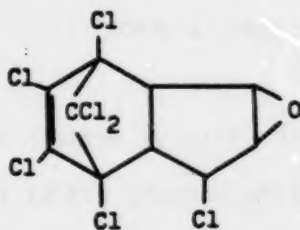
*Estimated from reverse phase HPLC data

The fate of heptachlor in aquatic media has been studied by several investigators. This compound may undergo significant photolysis in ambient aquatic media (Callahan et al., 1979). Verschueren (1983) reported that sunlight and artificial fluorescent light irradiation of 10 µg/l of this compound in a river water placed inside a sealed glass jar produced 75% decomposition in 1 week and complete decomposition in 2 weeks. From the estimated oxidation rate constant values given by Mabey et al. (1981), the singlet oxygen reaction with heptachlor in aquatic media may be significant. Based on an estimated rate constant of $3 \times 10^{-10} \text{ M}^{-1} \text{ hr}^{-1}$ (Mabey et al., 1981), and a concentration of 10^{-12} M for singlet oxygen in surface water (Mill et al., 1982), the half-life of heptachlor for this reaction is estimated to be ~1 day.

The hydrolysis of heptachlor in aquatic media is also an important process. It has been estimated that the hydrolytic half-life of heptachlor is in the range of 1-3 days (Callahan et al., 1979; Mabey et al., 1981). The volatilization half-life of heptachlor from aquatic media is estimated to be in the range of 2-10 days from pond, river and lake water. This estimate is based on the re-aeration rate ratio of 0.355 given by Mabey et al. (1981) and oxygen re-aeration rate of 0.19 day^{-1} , 0.96 day^{-1} and 0.24 day^{-1} for pond, river and lake water, respectively as given by Mill et al. (1982). Although the evaporative half-life of heptachlor in aquatic media appears to be longer than that of chlordane (2-10 days vs. 28-33 hours) from this estimate, Huang (1970) reported that the evaporation rate of heptachlor from aquatic media is faster than chlordane.

From the sediment-water sorption coefficient value of 1.2×10^{-4} given by Mabey et al. (1981), heptachlor may be significantly sorbed onto suspended particles and sediments present in aquatic media. Therefore, sorption may play a significant role in determining the fate of heptachlor in aquatic media (Callahan et al., 1979). In the treatment of wastewater, 5.9 mg/l carbon dose was required to reduce the pollutant concentration from 0.1 to 0.01 mg/l (U.S. EPA, 1981). The biodegradation rate of heptachlor in aquatic media is slower than the rate of hydrolysis (Mabey et al., 1981; Callahan et al., 1979).

Heptachlor Epoxide



Molecular formula: $C_{10}H_5Cl_7O$

Molecular weight: 389.32

RTECS number: PB 94500

Chemical Abstracts Service number: 1024-57-3

Heptachlor epoxide is the common name for 2,3,4,5,6,7,7-heptachloro-1a,1b,5,5a,6,6a-hexahydro-2,5-methano-2H-indene(1,2-b) oxirene. This chemical is also known as epoxyheptachlor; velsicol 53-CS-17; ENT 25, 584; 1,4,5,6,7,8,8-heptachloro-2,3-epoxy-3a,4,7,7a-tetrahydro-4,7-methanoindan. Heptachlor epoxide is not commercially available in the United States, but it is a degradation product of heptachlor (IARC, 1979b; Verschueren, 1983).

The chemical and physical properties of heptachlor epoxide are given in Table II-3.

The fate of heptachlor epoxide in aquatic media has not been comprehensively studied. From the information available in the reviewed literature, photolysis, oxidation or hydrolysis are not expected to be a significant fate-determining process for this chemical in aquatic media (Mabey et al., 1981; Callahan et al., 1979; Verschueren, 1983). From the estimated biotransformation rate constant ($3 \times 10^{-5} \text{ mL cell}^{-1} \text{ hr}^{-1}$) given by Mabey et al. (1981), and the estimated microbial population in surface water ($5 \times 10^5 \text{ cell mL}^{-1}$) given by Burns et al. (1981), the biodegradation of this compound does not appear to be a significant process.

The two processes that may determine the fate of heptachlor epoxide in aquatic media are volatilization and sorption. Huang (1970) reported that the rate of volatilization of heptachlor epoxide from aquatic media is much slower compared with heptachlor. However, it is difficult to make a reasonable estimate of the evaporative half-life for this compound because of the lack of appropriate data. The sorption of this compound on suspended particulate matter or sediment may be a moderately significant process (Mabey et al., 1981; Callahan et al., 1979). In the treatment of wastewater, a 2.2 mg/l carbon dose was required to reduce the concentration of this compound from 0.1 to 0.01 mg/l (U.S. EPA, 1981).

TABLE II-3

Chemical and Physical Properties of Heptachlor Epoxide

Property	Value or Description	Reference
Melting point	160-161.5°C (99.5% pure)	IARC, 1979b
Vapor pressure (estimated)	3×10^{-4} mm of Hg at 25°C	Mabey et al., 1981
Solubility	0.35 mg/l at 25°C	Mabey et al., 1981
Log octanol/water* partition coefficient	5.40 4.43 2.65	Mackay, 1982 Geyer et al., 1982 Mabey et al., 1981
Conversion factor	1 ppm = 15.9 mg/m ³ at 25°C and 760 mm of Hg	

*The difference in these calculated values is due to the different methods used for estimating the partition coefficient.

Summary

Chlordane and heptachlor are structurally related chlorinated hydrocarbon insecticides that have been and/or are being used to control pests on field crops and termites. Heptachlor epoxide is a degradation product of heptachlor. Technical grade chlordane is a yellow-colored liquid consisting of approximately equal parts cis- and trans-isomers, along with chlordane isomers, heptachlor and their impurities. It boils at 175°C at 2 mm Hg (Callahan et al., 1979), melts at 104-107°C (BCPC, 1977), has a vapor pressure of 1×10^{-5} mm Hg at 25°C (IARC, 1979a) and has a low water solubility but is soluble in most hydrocarbon solvents (Deichmann, 1981). Chlordane has a log octanol/water partition coefficient of 3.32 (Rao and Davidson, 1982).

Technical grade heptachlor is approximately 73% heptachlor and 22% transchlordane. The boiling point of the pure chemical (a white crystalline solid) is 135-145°C at 1-1.15 mm Hg; its melting point is 93°C and its vapor pressure is 3×10^{-4} mm Hg at 25°C (IARC, 1979b). Like chlordane, it has a low water solubility but is soluble in organic solvents. Literature values for log octanol/water partition coefficient were reported as 3.87 (Rao and Davidson, 1982) and 5.44 (Mackay, 1982) for heptachlor.

The melting point of pure (99.5%) heptachlor epoxide is 160-161.5°C (IARC, 1979b) and has an estimated vapor pressure of 3×10^{-4} mm Hg at 25°C (Mabey et al., 1981). It is slightly soluble in water (Mabey et al., 1981) and has a log octanol/water partition coefficient in the range of 2.65-5.40 (Mackay, 1982; Geyer et al., 1982; Mabey et al., 1981).

Neither chlordane nor heptachlor epoxide will undergo significant chemical reactions in drinking water (Callahan et al., 1979; Mabey et al., 1981). Volatilization of both chlordane and heptachlor epoxide is possible during aeration of drinking water; however, the rate of volatilization is faster for chlordane than heptachlor epoxide (Huang, 1970). Both compounds may be sorbed onto organic particulate matters and may be removed during the filtration processes. The rate of sorption with chlordane is higher than heptachlor epoxide (Mabey et al., 1981; Callahan et al., 1979). Heptachlor, on the other hand, may hydrolyze in drinking water (Mabey et al., 1981; Callahan et al., 1979) and may undergo oxidation with singlet oxygen (Mabey et al., 1981) during the water treatment process. Volatilization of heptachlor is possible during aeration of drinking water at a rate faster than that of either chlordane or heptachlor epoxide (Huang, 1970). Heptachlor may be sorbed onto organic particulate matter (Mabey et al., 1981; Callahan et al., 1979) and may be removed during filtration of drinking water.

III. TOXICOKINETICS

Animal Studies

Chlordane.

Absorption -- Quantitative data on the absorption of chlordane from the gastrointestinal tract of animals are not available; however, the systemic toxicity of the insecticide to laboratory animals following oral exposure (Chapter V) indicates that some absorption does take place. That chlordane is absorbed can also be inferred from excretion data. As discussed in Chapter II, chlordane consists of a mixture of components, including the cis- and trans-isomers. A high purity (98+%) formulation of the insecticide, designated HCS-3260, contains cis- and trans-chlordane in a 3:1 ratio. Sprague-Dawley rats (one of each sex) received a single dose of 0.05, 0.2 or 1.0 mg ^{14}C -HCS-3260/kg bw by gavage, or a single oral dose of each ^{14}C -labeled isomer at 0.2 mg/kg bw (Barnett and Dorough, 1974). Elimination of radioactivity in the urine over 7 days was 6% for females and 2% for males following ^{14}C -HCS-3260 treatment. Following treatment with cis- and trans-chlordane- ^{14}C , female rats eliminated 8.5 and 5% of the administered radioactivity, respectively. These results indicate that at least 2-8.5% of the administered chlordane dose was absorbed by the gastrointestinal tract of rats. In contrast, a male rabbit that was given ^{14}C -HCS-3260 in a dietary concentration of 25 ppm (25 mg/kg diet) for 2 days excreted 33% of the radioactivity in the urine and 21% in the feces 24 hours after termination of the treatment (Barnett and Dorough, 1974).

Pulmonary absorption of an unspecified amount of ^{14}C -chlordane (11,500 dpm/ μg) in 20 μl ethanol administered as an aerosol intratracheally to female Sprague-Dawley rats was observed by Nye and Dorough (1976). No

intact ^{14}C -chlordane was detected in exhaled air. A peak blood concentration of radioactivity of ~4% of the applied dose was reached in <5 minutes.

Ambrose et al. (1953b) reported in an abstract that dermal application of 50 mg chlordane/kg bw was more toxic to rats when the chemical was applied in cottonseed oil rather than ethyl alcohol, indicating greater absorption with the lipophilic vehicle.

Distribution -- The tissue distribution of ^{14}C -HCS-3260, cis- or trans-chlordane- ^{14}C and the metabolite, oxychlordane, in male and female rats following treatment with single oral doses was compared by Barnett and Dorrough (1974). At one day following doses of 0.05-2.0 mg/kg bw of the respective compounds, the concentrations of radioactive equivalents in brain, muscle, liver and kidney were generally low (0.00-0.08 ppm), while the concentrations of radioactivity in fat were somewhat higher (average for all treatments ~0.47 ppm). Male and female rats treated with 0.1 mg ^{14}C -HCS-3260/kg bw had higher tissue residue levels of radioactive equivalents in liver (0.50 ppm), kidney (0.26 ppm) and fat (3.71 ppm for females, 2.58 ppm for males). In general, female rats accumulated greater concentrations of radioactivity in fat than male rats after treatment with any preparation. At 7 days after dosing with 1.0 mg ^{14}C -HCS-3260/kg bw, radioactivity in all tissues declined; radioactivity in fat declined to 2.0 ppm for females and 1.19 ppm for males. Slightly more radioactivity was present in rat tissues following oral doses of trans- than cis-chlordane- ^{14}C .

When ^{14}C -HCS-3260 was administered to male rats in the diet at 5 ppm (5 mg/kg diet) for 56 days, the tissue distribution of radioactive equivalents was 0.42, 0.91, 0.55, 0.68 and 14.73 ppm for muscle, brain, kidney, liver and fat, respectively (Barnett and Dorrough, 1974). After discontinuing treatment for 28 and 56 days, the concentration of radioactivity in fat declined to 3.67 and 2.49 ppm, respectively. Radioactivity was still detected in other tissues 56 days after treatment was terminated. Greater accumulation of radioactivity in all tissues resulting from trans- rather than from cis-chlordane- ^{14}C occurred in female rats treated with 25 ppm (25 mg/kg diet). Analysis of the nature of the radioactivity revealed that ~30-60% of the radiocarbon was associated with oxychlordane.

Ambrose et al. (1953) found that the perirenal fat of male rats contained 43, 41 and 81 ppm of chlordane residues following feeding for 5, 148 and 407 days, respectively, of a diet containing 320 ppm (320 mg/kg diet). The fat of female rats contained approximately twice the values for males.

Residues of parent isomers and oxychlordane in adipose tissue from male and female Holtzman rats maintained on diets containing 50-200 ppm (50-200 mg/kg diet) cis- or trans-chlordane, or 100 ppm (100 mg/kg diet) of fixed ratios of the isomers from 9:1 trans:cis to 1:9 or 50 ppm (50 mg/kg diet) technical chlordane for 15 days were determined by Street and Blau (1972). Adipose tissue of female rats fed trans-chlordane at 50, 100 and 200 mg/kg diet contained ~6, 10 and 23 $\mu\text{g/g}$ lipid of trans-chlordane, respectively. Much greater concentrations of oxychlordane than trans-chlordane were stored in adipose tissue by females (~104, 202 and 471 $\mu\text{g/g}$ lipid) and males (~5, 15 and 22 $\mu\text{g/g}$ lipid) after being fed trans-chlordane. The feeding of

cis-chlordane likewise resulted in greater fat storage of the metabolite than of the parent isomer in both male and female rats; however, the ratio oxychlordane:isomer was reduced. Female rats fed 50 ppm technical grade chlordane (50 mg/kg diet) stored ~7 times more oxychlordane than the parent compound in adipose tissue. The results of feeding ratios of cis- and trans-chlordane indicated that oxychlordane accumulation was additive for each isomer.

Polen et al. (1971) also detected oxychlordane in the fat depots of rats, dogs and pigs maintained on diets containing individual isomers or technical chlordane. Rats received dietary concentrations of cis-, trans- or technical grade chlordane of 0-150 ppm (0-150 mg/kg diet) for 1 year; dogs received technical grade chlordane at 3 or 30 ppm (3 or 30 mg/kg diet) for 2 years; pigs were fed diets containing the cis- or trans-isomer at 300 ppm (300 mg/kg diet) for 90 days. The respective levels of oxychlordane in the fat were 0.2-150, 3-24 and 36-90 ppm.

Rabbits received trans-chlordane- ^{14}C daily per os in doses of 14.3 mg/rabbit/day for 10 weeks (Poonawalla and Korte, 1971). Two weeks after treatment was discontinued, low levels of radioactivity were detected in kidney (0.05%), liver (0.52%), heart (0.09%), lung (0.04%), spleen (0.03%), testes (0.03%) and brain (0.04%). Higher levels were found in adipose tissue (2.59% in abdominal fat, 1.53% in subcutaneous fat) and muscle (5.68%). Barnett and Dorough (1974) found that the tissues of a rabbit fed ^{14}C -HCS-3260 (25 mg/kg diet for 2 days) contained ^{14}C -oxychlordane and ^{14}C -dichlorochlordene.

The distribution of radioactivity in the tissues of rabbits administered cis- or trans-chlordane-¹⁴C orally in four doses, one given every 4 days, of 100 mg in a capsule per rabbit was as follows: fat > kidney > muscle > liver > brain (cis-); kidney > fat > liver > muscle > brain (trans-) (Balba and Saha, 1978). The majority of the radioactivity in the tissues was associated with oxychlordane, regardless of which isomer was administered.

The mobilization of chlordane or its metabolites from adipose tissue of rats after food deprivation was studied by Ingle (1952). Chlordane was removed from the diet after 80 weeks maintenance of 16 rats on a diet containing chlordane at 150 ppm (150 mg/kg diet). At weekly intervals, two male and two female rats were fasted for 48 hours and observed for symptoms of toxicity. After 4 weeks, symptoms such as tremors and hyperactivity were no longer observed.

The distribution of radioactivity (expressed as % of administered radioactivity/tissue) in selected tissues of rats following the previously described intratracheal administration of ¹⁴C-chlordane (Nye and Dorough, 1976) was 23.9% in lungs, 19.6% in liver, 0.3% in kidney and 0.1% in the bladder and its contents.

Metabolism -- The metabolism of chlordane has been fairly well studied in rats and rabbits in vivo and in vitro. Oxychlordane was a metabolite of both cis- and trans-chlordane (Street and Blau, 1972). A metabolic pathway, based upon in vitro studies with rat liver homogenates, was proposed in

which either isomer is dehydrogenated to 1,2-dichlorochlordene with subsequent epoxidation to oxychlordane. In these experiments, trans-chlordane was converted to oxychlordane at a 7-fold greater rate than was the cis-isomer.

Barnett and Dorrough (1974) isolated seven radioactive metabolites (in addition to the respective unchanged parent compounds) in the feces of rats that had been administered cis- or trans-chlordane- ^{14}C or the 3:1 mixture of cis- and trans-chlordane, designated as ^{14}C -HCS-3260, either as single oral doses (0.2 mg/kg bw) or by continuous feeding (5 mg/kg diet of ^{14}C -HCS-3260 for 56 days or 25 mg/kg diet cis- or trans-chlordane- ^{14}C for 14 days). These metabolites, analyzed by TLC and GLC, were tentatively identified as hydroxychlordane; chlordene chlorohydrin; monochloro and dihydroxy derivatives of chlordane; cis- and/or trans-dihydroxychlordane derivatives; a trihydroxylated chlordene; and a conjugated form of a hydroxylated chlordane metabolite. No oxychlordane or dichlorochlordene was detected in feces; however, oral administration of oxychlordane resulted in fecal excretion of unchanged oxychlordane. The nature of urinary metabolites was essentially the same as fecal metabolites in rats fed HCS-3260 in dietary concentrations of 25 mg/kg diet: oxychlordane was also present. The 24-hour feces of a rabbit fed ^{14}C -HCS-3260 at 25 mg/kg diet for 2 days contained the same fecal metabolites found in the rat, although the amounts of unchanged isomers were greater. The urine of the rabbit contained a greater percentage of the conjugated hydroxylated metabolites than did the urine of rats.

In the previously described study in rabbits, Balba and Saha (1978) identified the following urinary metabolites of cis-chlordane: 1-hydroxy-2-chlorochlordene, trans-chlordene chlorohydrin and 1-hydroxychlordene. Urinary metabolites in rabbits of trans-chlordane were 1-hydroxy-2-chlorochlordene, 1,2-dichlorochlordene, trans-chlordene chlorohydrin and 3-hydroxychlordane.

The metabolism of cis- and trans-chlordane-¹⁴C was also studied in rats in vivo and in vitro by Tashiro and Matsumura (1977). Male rats maintained on diets containing cis- or trans-chlordane-¹⁴C in a concentration of 100 ppm (100 mg/kg diet) for 4 weeks excreted 13 metabolites from cis- and 14 metabolites from trans-chlordane in the feces. The fecal metabolites of both isomers, identified by TLC and GLC, included heptachlor, 1,2-dichlorochlordene, oxychlordane, 1-hydroxy-2-chlorochlordene, 1-hydroxy-2-chloro-2,3-epoxychlordene, chlordene chlorohydrin, monohydroxy dihydrochlordene, 1,2-dihydroxychlordene and trihydroxydihydrochlordene. These metabolites were found in different proportions, depending upon the administered isomer. The only urinary metabolite identified from both cis- and trans-chlordane was a glucuronide conjugate of 1-hydroxydihydrochlordene. The in vitro incubation of the isomers with rat liver microsomes and cofactors resulted in the same metabolites qualitatively.

Based upon these in vivo and in vitro results, Tashiro and Matsumura (1977) concluded that chlordane is dehydrogenated to dichlorochlordene, which is epoxidized at carbons 2 and 3 and hydroxylated at carbon 1 (Route A). An additional route whereby chlordane is hydroxylated at carbon 1 to yield 1-hydroxydihydrochlordene was also proposed (Route B). These pathways are illustrated in Figure III-1.

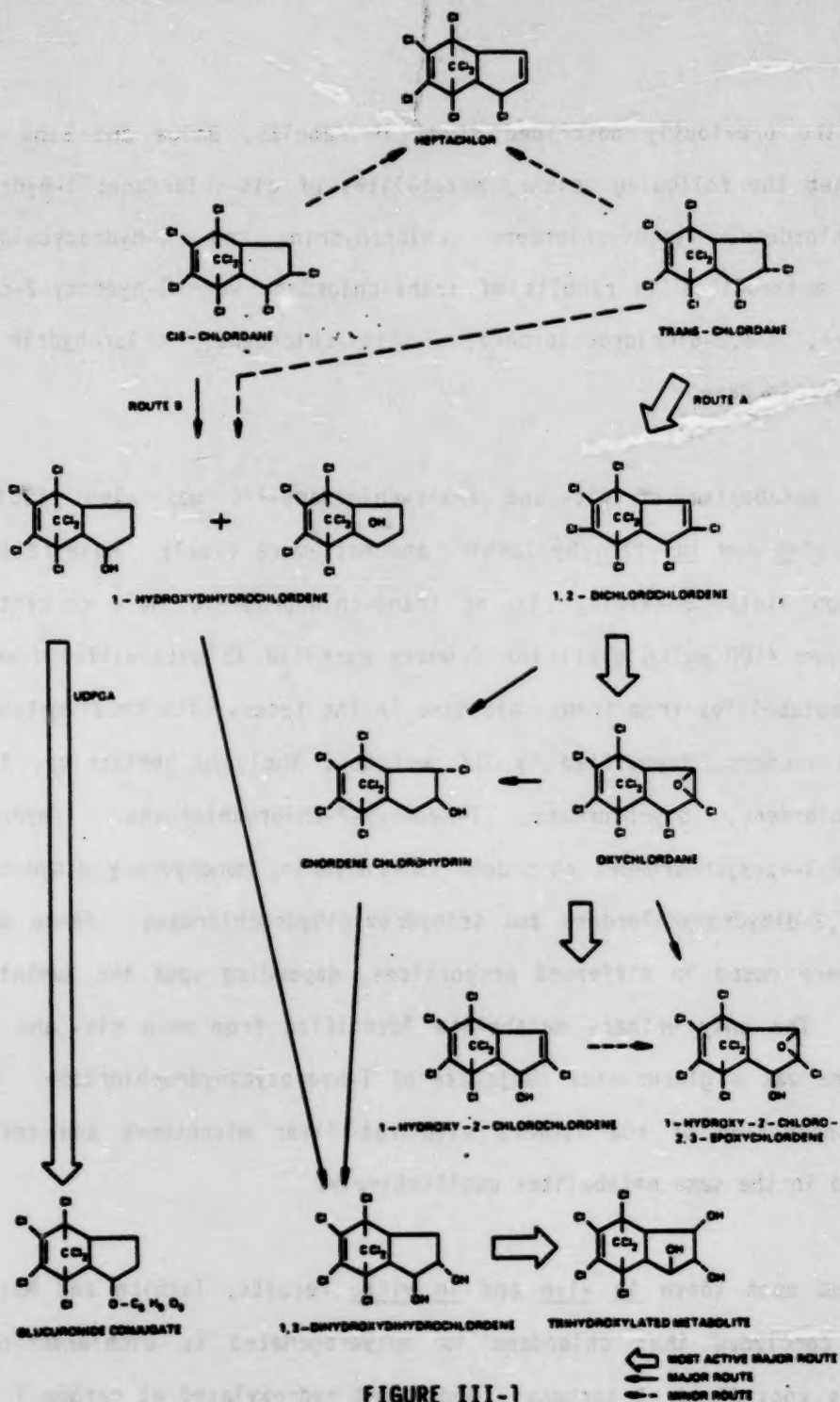


FIGURE III-1

Proposed Metabolic Pathways for Chlordane

Source: Tashiro and Matsumura, 1977

Brimfield et al. (1978) also studied the in vitro metabolism of the pure cis- and trans-isomers of chlordane by microsomal preparations from Sprague-Dawley rats fed the individual isomers in dietary concentrations of 100 ppm (100 mg/kg diet) for 9 days. Incubation of cis-chlordane-induced enzymes with cis-chlordane resulted in the following metabolites, identified by GC-MS: cis-chlordane, dichlorochlordene, oxychlordane, dihydroheptachlor, hydrochlordene and 1-chloro-2-hydroxydihydrochlordene. Similar products from trans-chlordene were identified; however, heptachlor rather than dihydroheptachlor was formed, as was hydroxychlordane. A metabolic pathway, based upon reductive dechlorination via dihydroheptachlor to dihydrochlordene, was proposed. According to this scheme, these molecules can also be hydrolyzed, desaturated and epoxidized. In the Brimfield et al. (1978) proposal, oxychlordane is an endpoint, whereas, in the scheme proposed by Tashiro and Matsumura (1977), oxychlordane undergoes further hydroxylation and reduction. Many of the proposed intermediates in the Brimfield scheme have not actually been found in this or other studies (Brimfield et al., 1978).

Excretion -- In the previously described experiments of Barnett and Dorough (1974), >90% of the administered radioactivity was excreted over 7 days by rats that were given single oral doses (0.2 mg/kg diet) of cis- or trans-chlordane- ^{14}C or ^{14}C -HCS-3260. Females excreted 6% of the radioactivity in the urine; males excreted 2%. At higher doses (0.5 or 1.0 mg/kg diet) of HCS-3260, the pattern of elimination was essentially the same. Females excreted slightly, but not significantly, more of the cis- than the trans-isomer. When ^{14}C -HCS-3260 was fed in the diet for 56 days, fecal elimination as measured by radioactivity was 70% for the 1 mg/kg diet level.

75% for the 5 mg/kg diet level and 80% for the 25 mg/kg diet level, indicating possible decreased absorption: the investigators made no mention of possible biliary excretion. Elimination of cis-chlordane (75%) was greater than trans-chlordane (65%) following 14 days of dietary levels of the isomers at 25 mg/kg diet.

Tashiro and Matsumura (1977) reported similar results for elimination from rats treated with single oral doses of cis- (5.4 mg/kg bw) and trans-chlordane- ^{14}C (9.7 mg/kg bw) in corn oil. The total 7-day elimination of radioactivity from cis- and trans-chlordane was 86 and 66% of the administered dose, respectively. The 24-hour total excretion was 59% for cis- and 27% for trans-chlordane.

Rabbits excreted 18% of an orally administered single dose of 200 mg/kg bw of chlordane in the urine collected over 16 days when organic chlorine content was measured (Stohlman et al., 1950). The peak urinary elimination of organic chlorine occurred within 2 days and amounted to 9% of the dose.

Poonawalla and Korte (1971) observed appreciable urinary excretion of radioactivity by rabbits that received 14.3 mg/rabbit of trans-chlordane- ^{14}C daily for 10 weeks. At the end of this period, ~70% of the daily dose had been eliminated: 22.7% was excreted in the feces, 30% of which was unchanged trans-chlordane; and 47% was excreted as urinary metabolites. In agreement with these results, excretion of radioactivity by one rabbit 24 hours after termination of feeding 25 ppm (25 mg/kg diet) of HCS-3260- ^{14}C for 2 days was 21% in feces and 33% in urine (Barnett and Dorough, 1974).

Balba and Saha (1978) also observed appreciable urinary excretion of radioactivity of cis- or trans-chlordane-¹⁴C by rabbits that were treated with either isomer orally at a dose of 100 mg/rabbit in a capsule every 4 days up to 400 mg/rabbit; however, in this study, urinary excretion did not exceed fecal excretion. For the cis-isomer, 48.5% and 28.4% of the radioactivity were eliminated in the feces and urine, respectively. For the trans-isomer, fecal and urinary excretion were 46.1% and 35.8%, respectively.

When an unspecified amount of chlordane-¹⁴C in 20 μ l of ethanol was administered intratracheally to female Sprague-Dawley rats, elimination of radioactivity was primarily in the feces (Nye and Dorough, 1976). After a lag period of 2 days during which <20% of the dose was eliminated, fecal excretion rose to ~50% by day 4 and 56% by day 6. Urinary excretion over 6 days amounted to 12% of the dose. No radioactivity was detected in the expired air of these animals.

Heptachlor and Heptachlor Epoxide.

Absorption -- Limited information on the absorption of heptachlor following ingestion by animals was available. The U.S. EPA (1980a) reviewed an abstract of a Russian study (Mizyukova and Kurchatov, 1970) and reported that heptachlor administered intragastrically in a single oral dose of 120 mg/kg bw to rats was detected in blood within 0.5-1 hour of administration.

The absorption of heptachlor following inhalation exposure also has not been well studied. From July 1, 1972 to October 4, 1972, Arthur et al. (1975) placed 10 rabbits of each sex in open-air cages so that they were

exposed to the ambient air of Stoneville, Mississippi, an area where insecticides had been heavily used. Control groups of male and female rabbits (10 each) were housed in a room at Mississippi State University, a low pesticide use area. The average air levels of heptachlor epoxide (heptachlor was either not measured or not detected) in Stoneville air was 1.86 ng/m³; the air at Mississippi State was not sampled. Heptachlor epoxide residue levels in adipose tissue of the test rabbits was 0.039 ppm compared with 0.016 ppm in controls ($p < 0.001$). The average respiratory intake of heptachlor epoxide was calculated as 0.002 µg/day for rabbits in the Stoneville area.

Distribution and Retention -- An abstract of a Russian study (Mizyukova and Kurchatov, 1970), reported that following a single intragastric dose of 120 mg/rat heptachlor to female rats, heptachlor was detected in blood, liver, kidney and adipose tissue within 1 hour. After 4 hours, heptachlor epoxide, a metabolite of heptachlor, was detected in blood, liver and fat and persisted in the adipose tissue for 3-6 months.

Radomski and Davidow (1953) studied the tissue distribution of heptachlor epoxide in rats and dogs following oral administration of heptachlor. Nine rats of either sex received dietary concentrations of heptachlor at 30-35 ppm (30-35 mg/kg diet) for 2 months. Heptachlor epoxide levels in the fat of six female rats averaged 384 µg/g tissue, while fat in male rats contained an average of only 43 µg/g tissue. Much lower levels of heptachlor epoxide were detected in liver (0.4-33 µg/g), kidney (0-21 µg/g) and muscle (0-27 µg/g). None was detected in brain tissue. A similar pattern of distribution was observed in three female dogs that received 1 mg

heptachlor/kg bw in capsules daily for 12-18 months. Fat contained an average heptachlor epoxide concentration of 636 $\mu\text{g/g}$; liver, 36 $\mu\text{g/g}$; kidney, 7.6 $\mu\text{g/g}$; and muscle, 13 $\mu\text{g/g}$. Again, no heptachlor epoxide was detected in the brain. Small amounts of unchanged heptachlor were detected in the fat of dogs when higher levels of the insecticide were administered. Administration of 1 mg heptachlor/kg bw to four dogs resulted in no detectable heptachlor in fat after 26 weeks, while three of eight dogs, surviving 6-18 weeks, dosed with 3 mg/kg bw daily had <1 μg heptachlor/g fat tissue. A daily dose of 5 mg/kg bw resulted in an average concentration of heptachlor in fat of 6.5 $\mu\text{g/g}$. This dose was fatal to the dogs within 2-11 weeks.

Radomski and Davidow (1953) also determined the rate of accumulation and disappearance of heptachlor epoxide in rats (three rats/sex/group) fed a diet containing 30 ppm (30 mg/kg diet) heptachlor. One group was sacrificed each week for 12 weeks. Female rats accumulated heptachlor epoxide to a maximum level of ~225 $\mu\text{g/g}$ fat in 8 weeks compared with only 50 $\mu\text{g/g}$ fat in males. When the test diet was discontinued, heptachlor epoxide was no longer detected at 8 weeks for female fat tissue and 6 weeks for male fat tissue. Further tests established that the maximum dietary level at which fat storage did not occur was 0.3 mg/kg diet for male rats and 0.1 mg/kg diet for female rats.

Although Radomski and Davidow (1953) failed to detect heptachlor epoxide in rat and dog brain tissue after heptachlor treatment, Yamaguchi et al. (1980), using a GC method, detected 3.15 ppm heptachlor epoxide in brain tissue of rats 5 hours after an intraperitoneal injection of 200 mg heptachlor/kg bw.

Metabolism -- Davidow and Radomski (1953) first identified heptachlor epoxide in the adipose tissue of dogs that had been treated with daily oral doses of 1-3 mg heptachlor/kg bw in corn oil. They concluded that the metabolite arose by the epoxidization of heptachlor. Subsequently, these investigators (Radomski and Davidow, 1953) also isolated heptachlor epoxide, identical to the compound found in dogs, from the adipose tissue of male and female rats maintained on diets containing 30-35 ppm (30-35 mg/kg diet) heptachlor.

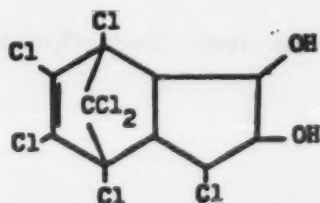
In an abstract of a Russian study, Ermakov (1977) reported that heptachlor, administered to rats and rabbits in single oral doses of 28-50 mg/kg bw, was metabolized by reactions involving hydrolysis, hydroxylation, epoxidation, dehydrogenation and molecular rearrangement, but details as to intermediate structure were not given in the abstract.

The most extensive investigation of the metabolic fate of heptachlor in rats was performed by Tashiro and Matsumura (1978) in vivo and in vitro. Over a 10-day period following a single oral unspecified dose of ¹⁴C-heptachlor (position of label not specified) in corn oil, rats excreted >50% of the administered radioactivity in feces, and <5% in urine. The relative abundance of fecal metabolites, expressed as percent of ¹⁴C-compounds, were as follows: unchanged heptachlor, 26.2%; heptachlor epoxide, 13.1%; 1-hydroxychlorde, 19.5%; 1-hydroxy-2,3-epoxychlorde, 17.5%; 1,2-dihydroxydihydrochlorde, 3.5%; and two other unnamed metabolites, one of which accounted for 19% of the radioactivity, the other for <0.1%. The latter metabolite was designated as H-2 by these authors, and its structure is identical to that proposed by Matsumura and Nelson (1971)

for what they called the fecal metabolite of heptachlor epoxide. The in vitro metabolism of ^{14}C -heptachlor by rat liver microsomal preparations resulted in the following relative abundance of metabolites, expressed as % of the total ^{14}C -compounds: heptachlor, 4.4%; heptachlor epoxide, 85.8%; 1-hydroxychlorde, 2.9%; 1-hydroxyepoxychlorde, 3.0%; 1,2-dihydroxydi-chlorde, 0.9%; and an unknown, 3.0% (Tashiro and Matsumura, 1978). No H-2 fecal metabolite was detected. The identity of the metabolites was confirmed by TLC analysis and comparison with authentic standards of fecal metabolites from rats fed diets containing 100 ppm (100 mg/kg diet) heptachlor for 4 weeks. A pathway of heptachlor metabolism by rats was proposed and is illustrated in Figure III-2.

Matsumura and Nelson (1971) administered heptachlor epoxide to four rats in dietary concentrations of 10 ppm (10 mg/kg diet) for 30 days. The authors estimated that each rat consumed 5 mg of heptachlor epoxide over the test period and excreted 950 μg of a fecal metabolite (see Figure III-2) and 66 μg of heptachlor epoxide in the feces.

Brooks et al. (1968, 1970) investigated the in vitro metabolism of heptachlor epoxide by pig liver microsomes. The product, formed upon incubation at 45°C for 60 hours, was identified as heptachlor epoxide diol:



Incubation of heptachlor epoxide with rabbit microsomes also resulted in the formation of heptachlor epoxide diol as well as another unidentified product.

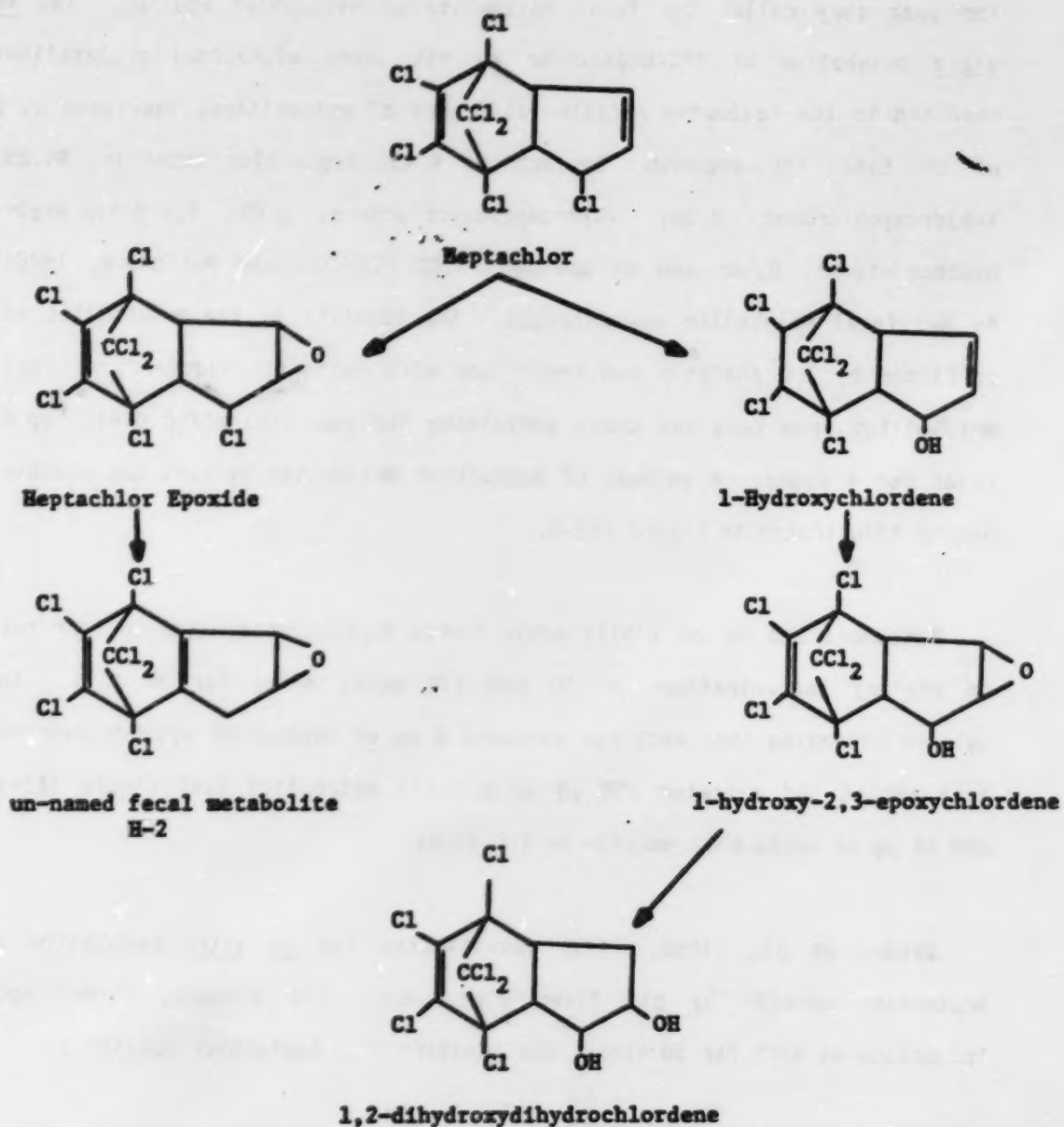


FIGURE III-2

Proposed Metabolic Pathway of Heptachlor in Rats

Source: Tashiro and Matsumura, 1978

Excretion -- Tashiro and Matsumura (1978) reported that rats excreted >50% of the administered single oral unspecified dose of radioactivity from ¹⁴C-heptachlor in the feces over 10 days. Urinary excretion of radioactivity accounted for <5% of the administered amount.

In an abstract of a Russian study (Ermakov, 1977), it was reported that 16-40% of the orally administered dose of heptachlor (28-50 mg/kg bw) to rats and rabbits was excreted unchanged in the feces and that heptachlor epoxide was excreted over at least a 12-month period. Further details were not given in the abstract. As reported in an abstract of another Russian study (Mizyukova and Kurchatov, 1970), and as reviewed by U.S. EPA (1980a), heptachlor and its metabolites were excreted mainly in the feces of rats within 5 days of an intragastric dose of 120 mg/kg bw.

Each of four male albino rats excreted an average of 950 µg of the fecal metabolite (see Figure III-2) of 99% pure heptachlor epoxide during 30 days of maintenance on a diet containing 10 ppm (10 mg/kg diet) heptachlor epoxide (each rat consumed ~5 mg over 30 days) (Matsumura and Nelson, 1971). Approximately 66 µg of unchanged heptachlor epoxide was also excreted in the feces.

Human Data

Chlordane.

Absorption -- A 20-month-old boy (12.7 kg) drank an unknown quantity of 74% technical grade chlordane from a bottle (Curley and Garrettson, 1969). He vomited 45 minutes later and convulsions began shortly thereafter. He was admitted to the hospital 75 minutes after ingestion of

the pesticide. The concentration of chlordane in a blood sample taken 2.75 hours after ingestion was 2.71 mg/l. The concentration in fat was 3.12 mg/kg bw. Thus, chlordane was absorbed from the gastrointestinal tract of this child. A 4-year-old girl (11 kg) drank an unknown amount of a 45% chlordane formulation (Aldrich and Holmes, 1969). She was admitted to the hospital 3 hours later, and gastric lavage was performed. The first serum sample taken (time not specified) contained 3.4 ppm (mg/kg serum), from which the investigators calculated an absorbed quantity of at least 1.62 mg.

Distribution and Retention -- Information on the distribution of chlordane to tissues following chlordane ingestion is available from a case report of accidental poisoning and from autopsy samples.

In the previously described case of the 20-month-old boy who drank an unknown quantity of 74% chlordane, Curley and Garrettson (1969), assuming that fat comprised 15% of the body weight, calculated that 5.9 mg chlordane was in the fat 3 hours after ingestion; 36 mg chlordane was present on the next day, and 65.0 mg chlordane was in fat 8 days after ingestion. Three months after ingestion, the concentration of chlordane in fat was 25.53 mg/kg. At this time the ratio of chlordane in fat to serum was 1470:1. No fat samples were collected by Aldrich and Holmes (1969) from the 4-year-old girl who ingested chlordane.

Biros and Enos (1973) reported that of 27 adipose tissue specimens obtained at autopsies from people of the general population, 21 were positive for oxychlordane, a metabolite of chlordane, with a mean concentration of 0.14 ± 0.09 ppm and a range of 0.03-0.40 ppm. The mean adipose tissue levels

of oxychlordanes in 10 autopsy samples from the Dade County, Florida, white female population in 1978 was 0.193 ppm, with a range of 0.07-0.49 ppm (Barquet et al., 1981). Cis- and trans-chlordane levels were below the limits of GC-electron capture detection (<0.10 ppm). Sovocool and Lewis (1975) also detected oxychlordanes in human adipose tissue obtained at autopsy from several hospitals.

Metabolism -- The in vitro metabolism of cis- and trans-chlordane-¹⁴C by rat and human liver microsomal preparations was compared by Tashiro and Matsumura (1978). Their data are presented in Table III-1. Examination of the table reveals very little interspecies difference between the rat and human in vitro metabolism of the two isomers.

Excretion -- In the case of accidental poisoning of a 4-year-old, 11 kg girl by ingestion of an unknown quantity of a 45% chlordane formulation (Aldrich and Holmes, 1969), a nonlinear decline of serum chlordane concentration from 3.4 to 0.138 mg/kg serum in the first 3 days and 0.03 mg/kg serum by 130 days was observed. During the last 90 days of this period, the concentration of chlordane in serum declined at a rate of 0.036 mg/kg day, and a biological half-life was estimated as 88 days. The chlordane concentration in urine declined from 1.93 to 0.05 ppm over the first 3 days after ingestion; however, 35 days later the urinary level was 0.13 ppm. The investigators speculated that the rise may have resulted from release of stored chlordane. On days 2 and 3 after ingestion, fecal chlordane levels were 719 and 105 ppm, respectively. No chlordane was detected in the feces 1 or 2 months later.

TABLE III-1

Metabolism of *cis*-Chlordane and *trans*-Chlordane *in vitro* by Human and Rat Liver Microsomal Preparations: The Data Are Expressed as Percent of the Total Starting Material^{a,b}

Starting Material and Metabolites	<i>cis</i> -Chlordane		<i>trans</i> -Chlordane	
	Human ^c	Rat	Human ^d	Rat
1,2-C1-chlordene	0.5	2.1	0.4	NA
Oxychlordane	1.0	5.2	0.4	2.3
<i>cis</i> -Chlordane	83.1	85.5	NA	NA
<i>trans</i> -Chlordane	NA	NA	85.25	86.4
C-4 (T-4) ^e	0.5	0.6	1.0	0.8
C-5 (T-5) ^f	0.1	0.1	0.2	0.1
HMG	0.6	NA	2.5	NA
Chlordane chlorohydrin	5.7	3.8	4.5	7.9
C-10 (T-10) ^h	2.9	0.9	0.8	1.3
1,2-OH-chlordene	0.4	0.3	0.4	0.3
C-12 (T-12) ⁱ	0.6	0.2	0.5	0.0
C-13 (T-13) ^j	4.6	1.3	3.5	0.8

^aSource: Tashiro and Matsumura, 1978

^bMicrosomal fraction prepared by centrifuging human liver tissue homogenate at 20,000 x g for 20 minutes. Incubation mixture made up to 5 ml with incubation at 37°C in shaking incubator for 2 hours.

^cPatient A only

^dAverage of two patients (A and B). The amounts of *trans*-chlordane remaining were 86.6% for A and 83.9% for B.

^eC-4, T-4 = 1-hydroxy-2-chlorochlordene derived from *cis*- or *trans*-chlordane

^fC-5, T-5 = 1-hydroxy-2-chloro-2,3-epoxychlordene

^gUnknown metabolite found only in human experiments

^hC-10, T-10 = monohydroxylated dihydrochlordene

ⁱC-12, T-12 = trihydroxydihydrochlordene

^jC-13, T-13 = beta-glucuronide-1-hydroxydihydrochlordene

NA = Not applicable

In the case of the 20-month-old boy who ingested an unknown quantity of 74% chlordane, Curley and Garrettson (1969) estimated the half-life of chlordane in serum to be 21 days. The concentration of chlordane in the 24-hour urine was 0.309 mg/l, corresponding to 48.8 µg chlordane. Further urine samples were not collected.

In a survey conducted in Arkansas and Mississippi in 1973 and 1974, Strassman and Kutz (1977) reported that of 57 human milk samples, 45.6% were positive (quantifiable) for oxychlordane residues, while 54.4% contained unquantifiable traces. The mean level of oxychlordane residues in positive samples was 0.012 ppm. Thus, a route of chlordane or oxychlordane excretion in human females is lactation. The investigators stated that, to their knowledge, this was the first report of oxychlordane occurrence in human milk.

Heptachlor and Hepachlor Epoxide.

Absorption -- No information on the absorption of heptachlor or heptachlor epoxide by humans was available. That these insecticides are absorbed can be inferred from the many reports of adipose tissue levels of heptachlor epoxide, although the route cannot be readily ascertained.

Distribution -- Klemmer et al. (1977) reported that heptachlor epoxide was detected in tissue samples from 77 autopsies performed from 1966-1968 in a range of concentrations of 1-32 ppb whole tissue. The highest concentrations occurred in bone marrow and liver.

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Heptachlor epoxide has been detected in human adipose tissue in a large number of surveys conducted over the past several years in Great Britain (Abbott et al., 1972; 1981), Brazil (Wassermann et al., 1972), Japan (Curley et al., 1973), Israel (Wassermann et al., 1974), Texas (Burns, 1974), Louisiana (Greer et al., 1980), and the continental United States (Kutz et al., 1979, Sovocool and Lewis, 1975).

Several studies have reported transplacental transfer of heptachlor or heptachlor epoxide in humans. Curley et al. (1969) detected heptachlor epoxide in adipose tissue, brain, adrenals, lungs, heart, liver, kidney and spleen of 10 stillborn babies and 2 babies who died soon after birth and in 27 of 30 samples of cord blood from healthy neonates. All concentrations fell within the range of levels detected in the general adult population. Others have also reported that heptachlor epoxide levels in tissues of stillborn and soon dead infants correspond to concentrations found in adults (Zavon et al., 1969; Wassermann et al., 1972, 1974). A comparison of heptachlor and heptachlor epoxide residues in 53 maternal blood and 54 placental tissue samples revealed that, in general, high levels of the compounds in maternal blood were reflected as high levels in placentae and vice versa (Selby et al., 1969). The ratio of the concentration of heptachlor epoxide in placentae to the concentration in maternal blood was 5.8:1, based upon geometric means. For total equivalents of heptachlor, defined as the sum of the values for heptachlor and heptachlor epoxide, the ratio was 8.8:1. Polishuk et al. (1977a) reported mean levels of heptachlor epoxide in the extracted lipid of tissues from 24 women during labor as follows: adipose tissue, 0.2856 ppm; maternal blood, 0.2798 ppm; fetal blood, 0.9959 ppm; uterine muscle, 0.4895 ppm; placenta, 0.5000 ppm; and amniotic fluid 0.6730 ppm.

Metabolism -- The results of a comparison of the in vitro metabolism of heptachlor by human and rat liver microsomal preparations (Tashiro and Matsumura, 1978) are presented in Table III-2. A major difference between the species is that 4 times more heptachlor epoxide was formed in the rat system than in the human system. The investigators pointed out that since heptachlor epoxide is a stable product not involved in the major metabolic pathway of heptachlor (see Figure III-2), the relative ease of its formation may influence the relative degree to which heptachlor and heptachlor epoxide are stored by adipose tissue.

Excretion -- Information regarding the urinary and fecal excretion of heptachlor and heptachlor epoxide by humans was not available. Excretion of heptachlor epoxide in the milk of lactating mothers, however, has been reported by several investigators. Kroger (1972) detected an average of 0.16 ppm heptachlor epoxide in 53 human milk samples. A mean concentration of 0.003 mg/kg of heptachlor epoxide in milk was reported by Ritcey et al. (1972) in 147 human milk samples collected in Canada in 1967-1968. Heptachlor epoxide, in trace amounts up to concentrations of 5 ppb, was detected in 10 of 40 milk samples collected from women in Colorado in 1971-1972 (Savage et al., 1973). The mean concentration of heptachlor epoxide in 18 of 50 human milk samples collected in Norway was 1.57 ppb (Bakken and Seip, 1976). In Israel in 1975, a mean concentration of 0.0091 ppm was detected in whole milk collected from 29 women 2-4 days after delivery (Polishuk et al., 1977b). Of 51 human milk samples collected in St. Louis, MO, in 1973, 12 were positive for heptachlor epoxide (mean concentration = 0.0027 ppm) and 3 were positive for heptachlor (mean concentration = 0.019 ppm) (Jonsson et al., 1977). Strassman and Kutz (1977) detected heptachlor epoxide in

TABLE III-2

Heptachlor Metabolism *in vitro* by Human and Rat Liver Microsomal Preparations: The Data Are Expressed as Percent of Starting Material*

Starting Material and Metabolites	Human	Rat
Heptachlor	68.6	4.4
H-1 (heptachlor epoxide)	20.4	85.8
H-2	<0.1	--
H-3 (1-OH-chlordene)	4.8	2.9
H-4 (1-OH-chlordene epoxide)	5.0	3.0
H-5 (1,2-OH-chlordene)	0.1	0.9
H-6	1.0	3.0

*Source: Tashiro and Matsumura, 1978

35.1% of 57 human milk samples from Arkansas and Mississippi in 1973-1974 in a mean concentration of 0.004 ppm. All milk samples from 50 lactating women in Hawaii collected from 1979-1980 contained heptachlor epoxide in a mean concentration of ~34 ppb (Takahashi et al., 1981). Lactation may, therefore, represent an important route of excretion of heptachlor epoxide in nursing mothers and reduce the body burden in this human subpopulation. On the other hand, breast feeding results in an additional source of pesticide exposure for infants.

Summary

Chlordane. The absorption of chlordane by the gastrointestinal tract of animals can be inferred from data on its systemic toxicity and from excretion data following ingestion. At least 2-8% of orally administered ^{14}C -chlordane was absorbed by rats and at least 30% was absorbed by rabbits, as evidenced by the percent radioactivity recovered in urine (Barnett and Dorough, 1974). Chlordane was absorbed via the pulmonary route by rats (Nye and Dorough, 1976). More chlordane in lipophilic vehicle than in ethanol was absorbed by rats dermally (Ambrose et al., 1953b). Information on the absorption of chlordane by humans following ingestion is available from case studies of accidental poisoning of children (Curley and Garrettson, 1969; Aldrich and Holmes, 1969).

Once absorbed, chlordane and/or its metabolites are rapidly distributed throughout the body with highest levels detected in adipose tissue (Barnett and Dorough, 1974). Radioactivity in all tissues declined when treatment was discontinued, but detectable levels were still present in all tissues of rats 56 days after termination of feeding ^{14}C -chlordane for 56 days.

Female rats accumulated greater concentrations of chlordane or metabolites than did male rats (Barnett and Dorough, 1974; Ambrose et al., 1953; Street and Blau, 1972). Greater accumulation of residues by rats from trans- than from cis-chlordane was observed (Barnett and Dorough, 1974; Street and Blau, 1972). The major form of chlordane stored in tissues has been identified as oxychlordane (Barnett and Dorough, 1974; Street and Blau, 1972; Polen et al., 1971). Similar results have been observed in dogs, pigs (Polen et al., 1971) and rabbits (Poonawalla and Korte, 1971; Barnett and Dorough, 1974) and in rats following pulmonary absorption (Nye and Dorough, 1976). Oxychlordane has been detected in general population human adipose tissue obtained at autopsy (Biros and Enos, 1973; Barquet et al., 1981; Sovocool and Lewis, 1975). Chlordane was stored for at least 3 months in the fat of one child who drank a formulation of the insecticide (Curley and Garrettson, 1969).

Oxychlordane is a metabolite of both cis- and trans-chlordane in rats in vivo and in vitro, although more oxychlordane is formed from trans-chlordane (Street and Blau, 1972; Barnett and Dorough, 1974; Tashiro and Matsumura, 1977; Brimfield et al., 1978). At least 13 or 14 fecal metabolites have been isolated, many of which have been identified; they are identical, whether formed from cis- or trans-chlordane. A urinary metabolite was found to be a glucuronide conjugate (Tashiro and Matsumura, 1977). A metabolic scheme involving dehydrogenation, epoxidation, hydroxylation and dechlorination reactions was presented. Metabolism of chlordane in vitro by human liver microsomal preparations was found to be very similar to rat microsomal in vitro metabolism (Tashiro and Matsumura, 1978).

The major route of elimination of chlordane and its metabolites by rats following ingestion is via the feces, amounting to as much as 88% of the administered dose (Barnett and Dorough, 1974). The cis-isomer was excreted to a greater extent than was the trans-isomer (Barnett and Dorough, 1974; Tashiro and Matsumura, 1977). Since more oxychlordane, which is stored in adipose tissue, is formed from trans- than from cis-chlordane, the greater elimination of the cis-isomer is not surprising. Female rats excreted a greater percentage of metabolites in urine than did males (Barnett and Dorough, 1974). Urinary excretion of metabolites by rabbits was much greater than by rats, and in some cases exceeded fecal excretion of metabolites by rabbits (Poonawalla and Korte, 1971; Barnett and Dorough, 1974; Balba and Saha, 1978). Elimination of chlordane or metabolites by the pulmonary route was not observed in rats (Nye and Dorough, 1976). Excretion of chlordane by a 4-year-old girl was nonlinear, with a calculated half-life in serum of 88 days (Aldrich and Holmes, 1969). Urinary excretion declined for 3 days after ingestion, but rose at 35 days possibly as a result of a release of stored chlordane. A half-life of chlordane in serum of 21 days was calculated for a 2-year-old boy who drank chlordane (Curley and Garrettson, 1969). Oxychlordane has been found in the milk of lactating women (Strassman and Kutz, 1977).

Heptachlor and Heptachlor Epoxide. Heptachlor was absorbed from the gastrointestinal tract of rats following intragastric administration, as evidenced by the appearance of residues in blood within 1 hour of oral dosing (Mizyukova and Kurchatov, 1970), and by the pulmonary route by rabbits that breathed ambient air in a heavy pesticide use area, as evidenced by increased levels of heptachlor epoxide in adipose tissue over

control levels (Arthur et al., 1975). Absorption of heptachlor by humans can be inferred from many reports of levels of heptachlor epoxide in adipose tissue obtained in autopsy and biopsy samples from the general population (Klemmer et al., 1977; Abbott et al., 1972; 1981; Wassermann et al., 1972; 1974; Curley et al., 1973; Burns, 1974; Greer et al., 1980; Kutz et al., 1979). Heptachlor epoxide has been detected in all monitored tissues of deceased infants, and in cord blood of healthy neonates at levels corresponding to levels in the general adult population (Curley et al., 1969; Zavon et al., 1969; Wassermann et al., 1972, 1974). Following absorption of heptachlor, its metabolite, heptachlor epoxide, is distributed throughout the body of rats and dogs (Mizyukova and Kurchatov, 1970; Radomski and Davidow, 1953). Female rats accumulated 5-10 times as much heptachlor epoxide in adipose tissue as did males following continuous feeding of heptachlor for 8 weeks. Heptachlor epoxide persisted in fat of female rats for 8 weeks and male rats for 6 weeks after treatment was terminated. The maximum dietary level at which fat storage could not be detected was 0.3 mg/kg diet for male rats and 0.1 mg/kg diet for female rats. Heptachlor was metabolized by rats in vivo and in vitro to heptachlor epoxide, which was dichlorinated and hydroxylated to a fecal metabolite (Tashiro and Matsumura, 1978). Heptachlor was also metabolized to 1-hydroxychlordehene, which was epoxidized to 1-hydroxy-2,3-epoxychlordehene; 1-hydroxy-2,3-epoxychlordehene was hydrated to 1,2-dihydroxydihydrochlordehene. As much as 4 times more heptachlor epoxide was formed in vitro by rat microsomes than by human microsomes; otherwise, metabolism was similar (Tashiro and Matsumura, 1978).

Like chlordane, the major route of heptachlor elimination by rats is via the feces, amounting up to 50% over 10 days of the administered oral dose (Tashiro and Matsumura, 1978). Urinary excretion of metabolites amounted to <5% of the dose. The only information available on human excretion of heptachlor are reports of heptachlor epoxide detected in milk of lactating women (Kroger, 1972; Ritcey et al., 1972; Savage et al., 1973; Bakken and Seip, 1976; Polishuk et al., 1977b; Jonsson et al., 1977; Strassman and Kutz, 1977; Takahashi et al., 1981). This excretion reduced the body burden of heptachlor epoxide in these women but increases the exposure of breast-fed infants.

IV.A. HUMAN EXPOSURE

Humans may be exposed to chemicals such as heptachlor and heptachlor epoxide from a variety of sources, including drinking water, food, ambient air, occupational settings and consumer products. This analysis of human exposure to heptachlor and heptachlor epoxide is limited to drinking water, food, and ambient air because those media are considered to be sources common to all individuals. Even in limiting the analysis to these three sources, it must be recognized that individual exposure will vary widely based on many personal choices and on several factors over which there is little control. Where one lives, works, and travels, what one eats and physiologic characteristics related to age, sex and health status can all profoundly affect daily exposure and intake. Individuals living in the same neighborhood or even in the same household can experience vastly different exposure patterns. In the Exposure Estimation section of this chapter, available information is presented on the range of human exposure and intake for heptachlor and heptachlor epoxide from drinking water, food and ambient air for the 70-kg adult male. It is not possible to provide an estimate of the number of individuals experiencing specific combined exposures from those three sources. However, the Summary section of this chapter provides some insight into the relative contributions of the three sources, especially drinking water, to the range of intake values suggested by the available data.

Exposure Estimation

Drinking Water. No Federal surveys were obtained that included heptachlor and heptachlor epoxide as analytes. Several studies at the regional level have addressed concentrations of heptachlor and heptachlor epoxide in

drinking water, and are presented below. Irwin and Healy (1978) summarized data collected in 1976 by the USGS and the Florida Department of Environmental Regulation during a water quality reconnaissance of public water supplies in Florida. The study was conducted to determine the background concentrations of a variety of pollutants, including heptachlor and heptachlor epoxide. None of 16 surface water supplies sampled or 100 groundwater supplies sampled using the five aquifers in Florida contained either chemical in excess of the detection limits. The detection limits were not reported. Sandhu et al. (1978) summarized the results from a rural water supply study in Hampton and Chesterfield counties in South Carolina. In this groundwater study the number of samples taken and the number of positive samples were not reported. However, the percent of samples exceeding the detection limit of 0.01 $\mu\text{g/l}$ was reported. The results showed that 62.5% of the samples taken in Chesterfield County had detectable levels of heptachlor and 42% had detectable levels of heptachlor epoxide. For the Chesterfield County samples, the sample means were 0.015 and 0.008 $\mu\text{g/l}$ for heptachlor and heptachlor epoxide, respectively. In Hampton County, 45.5% of the samples contained heptachlor and 64% of the samples contained heptachlor epoxide in excess of the detection limit. The sample means for the Hampton county samples were 0.009 and 0.018 $\mu\text{g/l}$ for heptachlor and heptachlor epoxide, respectively.

Pellizzari et al. (1979) presented monitoring data for halogenated hydrocarbons in drinking water from the northern New Jersey area and the Buffalo/Niagara area. The limits of detection ranged from 0.005-0.025 $\mu\text{g/l}$ for both analyses. Of 22 drinking water samples taken from the northern New Jersey area, 2 samples (9%) contained 0.007 and 0.02 $\mu\text{g/l}$

heptachlor and none contained heptachlor epoxide. None of the 16 drinking water samples from the Buffalo/Niagara area were found to contain heptachlor or heptachlor epoxide in excess of the detection limits. The water source was not specified in this study. In a report on source identification of pollutants entering a sewage treatment plant, Levins et al. (1979) sampled two surface water sources in a drainage basin in Georgia. Although detection limits were not reported, heptachlor and heptachlor epoxide were not detected in either of the drinking water samples. Tucker and Burke (1978) reported that a concentration of heptachlor epoxide of 0.6 $\mu\text{g}/\text{l}$ was found in groundwater sampled from a public drinking water supply well in Camden County, NJ. The well was removed from service. The levels of heptachlor and heptachlor epoxide observed in drinking water, when they have been found, are in the very low $\mu\text{g}/\text{l}$ range. Assuming that heptachlor epoxide is present at the highest concentration reported, i.e., 0.6 $\mu\text{g}/\text{l}$ in Camden County, NJ, the daily intake for a 70-kg adult male consuming 2 l of drinking water/day would be 1.2 $\mu\text{g}/\text{day}$ or 0.017 $\mu\text{g}/\text{kg}/\text{day}$.

Diet. Information on the dietary intake of heptachlor was not obtained. The Food and Drug Administration's (FDA) Market Basket Studies for fiscal years 1975 through 1979 reported that heptachlor was "not detected" in any of the food samples analyzed in both adult and infant/toddler surveys. Limited information was obtained on dietary exposure to heptachlor epoxide. Dietary heptachlor epoxide intake has been reported for adults, as well as infants and toddlers, in the FDA Market Basket Studies for fiscal year 1979 (FDA, 1982a,b). Total dietary intake of heptachlor epoxide for adults in FY79 was 0.397 $\mu\text{g}/\text{day}$ (FDA, 1982a). The adult dietary intake included 0.255 $\mu\text{g}/\text{day}$ from dairy products; 0.130 $\mu\text{g}/\text{day}$ from meat, fish

and poultry; 0.006 $\mu\text{g/day}$ from potatoes; 0.002 $\mu\text{g/day}$ from root vegetables; and 0.004 $\mu\text{g/day}$ from oils and fats. Assuming that the average adult male weighs 70 kg, an intake of 0.397 $\mu\text{g/day}$ would be equivalent to 0.0057 $\mu\text{g/kg/day}$. [Note: The FDA study reported dietary intake data for a 69.1 kg adult male. The calculations made for this section (as well as for air and drinking water) are based on a 70-kg adult male, which is used as a reference body weight by the Office of Drinking Water.] Given the lack of additional data, the dietary intake of heptachlor epoxide was assumed to be at that level. The relative daily intakes of heptachlor epoxide are presented for FY74 through FY79 in Table IV.A-1. No trend in dietary intake levels is apparent for the years studied.

Total dietary intakes of heptachlor epoxide for infants and toddlers in FY79 were 0.172 and 0.245 $\mu\text{g/day}$, respectively (FDA, 1982b). These levels included: 0.156 $\mu\text{g/day}$ for whole milk, 0.013 $\mu\text{g/day}$ for other dairy and substitutes and 0.004 $\mu\text{g/day}$ for meat, fish and poultry in the infant study; 0.111 $\mu\text{g/day}$ for whole milk, 0.057 $\mu\text{g/day}$ for other dairy and substitutes, 0.077 $\mu\text{g/day}$ for meat, fish and poultry and 0.001 $\mu\text{g/day}$ for oils and fats in the toddler study. (The individual intake levels may not add to the total intake due to rounding). Assuming that the average infant weighs 8.2 kg (6-month-old) and the average toddler (2-year-old) weighs 13.7 kg, intakes of 0.172 $\mu\text{g/day}$ for the infant and 0.245 $\mu\text{g/day}$ for the toddler would be equivalent to 0.0210 and 0.0179 $\mu\text{g/kg/day}$, respectively. The infant and toddler intake levels for FY79 may be compared to the calculations of intake made by FDA for FY75 through FY78, which are presented below. Recent dietary intake values for infants/toddlers are higher than in the earlier studies.

TABLE IV.A-1

Estimated Dietary Intake of Heptachlor Epoxide for
Adults, Infants and Toddlers ($\mu\text{g/kg/day}$)*

	Adult	Infant	Toddler
FY79	0.0057	0.0210	0.0179
FY78	0.0077	0.0230	0.0188
FY77	0.0074	0.0133	0.0182
FY76	0.0055	0.0001	0.0057
FY75	0.0072	0.0097	0.0057
FY74	0.0092	--	--

*Source: FDA 1980a,b; 1982a,b

Assumptions: 70 kg adult male, 8.2 kg infant,
13.7 kg toddler.

-- = Data not obtained.

FDA comparisons for both FY77 and FY79 (FDA, 1980b; 1982a,b) of estimated intakes of heptachlor epoxide by geographic region show that the intakes for infants and toddlers were highest in the North Central region. In FY77, the average infant intake was $\sim 0.013 \mu\text{g/kg/day}$, while the infant intake for the North Central region was $0.037 \mu\text{g/kg/day}$. For toddlers, the average intake was $0.018 \mu\text{g/kg/day}$ and the estimate for the North Central region was $0.030 \mu\text{g/kg/day}$. In FY79, the average infant intake was $\sim 0.021 \mu\text{g/kg/day}$, and the infant intake for the North Central region was $0.041 \mu\text{g/kg/day}$. The average intake for toddlers was 0.018 , with an estimate for the North Central region of $0.051 \mu\text{g/kg/day}$. Only nominal differences were observed in comparisons of adult intakes by geographic region for FY79. Geographic data for adult intakes in FY77 were not obtained.

Air. Several studies have been performed by EPA in recent years to determine levels of pesticides in ambient air. Kutz et al. (1976) reported on levels of heptachlor in the ambient air of 16 states between 1970 and 1972. The maximum level measured was 27.8 ng/m^3 in Tennessee; the mean value for all positive samples was 1.0 ng/m^3 . Heptachlor was detected in 42% of the 2479 samples collected during the monitoring period (no detection limit was reported). Although the frequency of detection increased slightly during the study period (38-44%), the mean of positive samples declined from $1.4\text{--}0.7 \text{ ng/m}^3$. Kutz et al. (1976) cautioned that the sampling locations in these studies were selected for potentially high concentrations of pesticides in ambient air.

Kutz et al. (1976) also presented the results of a pilot suburban air monitoring program that was conducted between April 1975 and June 1975. During that period, five duplicate samples were collected in suburban areas of Miami, Florida; Jackson, Mississippi; and Fort Collins, CO. Heptachlor was detected in all of the samples collected near Miami and Jackson at mean values of 2.1 and 11 ng/m³, respectively. None of the samples collected from Fort Collins, CO, contained detectable levels of heptachlor (no detection limit was reported). The maximum value of heptachlor measured in the 30 samples was 22.1 ng/m³. Heptachlor epoxide was detected in 20% of the samples from Jackson, MS, with a maximum reported value of 1.3 ng/m³. No heptachlor epoxide was detected in samples from Florida or Colorado. Arthur et al. (1976) collected and analyzed air samples from the Mississippi Delta, one of the highest pesticide usage areas in the United States due to the intensive cotton production. Between 1972 and 1974, samples were collected at weekly intervals; a total of 156 samples were obtained. The maximum heptachlor and heptachlor epoxide levels reported were 0.8 and 9.3 ng/m³, respectively. No other information was reported regarding levels of pesticides monitored during the sampling period. The daily respiratory intake of heptachlor epoxide from air was estimated using the assumptions presented in Table IV.A-2 and a range of levels for heptachlor epoxide based on the monitoring data presented above. The estimates in Table IV.A-2 indicate that the daily heptachlor epoxide intake from air for adults exposed to the two levels ranges from 0.023-0.23 µg/day (0.0003-0.003 µg/kg/day). The values presented do not account for variances in individual exposure or uncertainties in the assumptions used to estimate exposure.

TABLE IV.A-2

Estimated Respiratory Intake of
Heptachlor Epoxide

Exposure ($\mu\text{g}/\text{m}^3$)	Intake	
	($\mu\text{g}/\text{day}$)	($\mu\text{g}/\text{kg}/\text{day}$)
0.001	0.023	0.0003
0.01	0.23	0.003

Assumptions: 70-kg man, 23 m³ of air inhaled/
day (ICRP, 1975).

Summary

Table IV.A-3 presents a general view of the total amount of heptachlor epoxide potentially received by a 70-kg adult male from air, food and drinking water. Six separate exposure levels for drinking water and two levels each for food and air (representing low and high levels of a probable range) are shown in the table. The relative source contribution data are presented for heptachlor epoxide because heptachlor, the manufactured chemical, tends to undergo biotransformation to heptachlor epoxide once it enters the environment. The data presented have been selected from an infinite number of possible combinations of concentrations for the three sources. Whether exposure occurs at any specific combination of levels is not known; nor is it possible to determine the number of persons that would be exposed to heptachlor epoxide at any of the combined exposure levels. The data presented represent possible exposures based on the occurrence data and the estimated intakes.

The values presented in Table IV.A-3 for ambient air levels of heptachlor epoxide, as well as the values for food levels, represent a high and low from the range of values found in the heptachlor epoxide monitoring data (see Diet and Air). Similarly, the levels presented for drinking water are representative of the monitoring data on heptachlor epoxide in drinking water (see Drinking Water). The values are 0.005 and 0.01 $\mu\text{g/kg/day}$ for food, and 0.001 and 0.01 $\mu\text{g/m}^3$ for ambient air. Assuming an air level between 0.001-0.01 $\mu\text{g/m}^3$ and the estimated heptachlor epoxide intake between 0.005-0.01 $\mu\text{g/kg/day}$ from foods, drinking water would be the predominant source of heptachlor epoxide exposure in the adult male only at drinking water levels between 0.19-0.46 $\mu\text{g/l}$. An accurate assessment of

TABLE IV.A-3

Estimated Intake of Heptachlor Epoxide from the Environment by Adult Males in $\mu\text{g/kg/day}$

Concentration Drinking Water ($\mu\text{g/l}$)	Low Intake Levels for Food and Air and Air Sources (Food dose: 0.005 $\mu\text{g/kg/day}$; Air Dose: 0.0003 $\mu\text{g/kg/day}$)				High Intake Levels for Food and Air Sources (Food Dose: 0.01 $\mu\text{g/kg/day}$; Air Dose: 0.003 $\mu\text{g/kg/day}$)			
	Total Intake From Environment	% From* Drinking Water	% From* Food	% From* Air	Total Intake From Environment	% From* Drinking Water	% From* Food	% From* Air
0.0	0.0053	0	94	6	0.013	0	77	23
0.005	0.0054	2	93	6	0.013	1	76	23
0.01	0.0056	5	89	5	0.013	2	75	23
0.02	0.0059	10	85	5	0.014	4	74	22
0.1	0.0083	36	60	4	0.016	19	63	19
0.6	0.022	76	22	1	0.03	57	33	10

*Numbers may not add to 100% due to rounding.

Assumptions: 70-kg adult male, 23 m^3 of air inhaled/day and 2 L of water consumed/day.

Intake from each source:

Water: 0.005 $\mu\text{g/l}$: 0.0001 $\mu\text{g/kg/day}$
 0.01 $\mu\text{g/l}$: 0.0003 $\mu\text{g/kg/day}$
 0.02 $\mu\text{g/l}$: 0.0006 $\mu\text{g/kg/day}$
 0.1 $\mu\text{g/l}$: 0.003 $\mu\text{g/kg/day}$
 0.6 $\mu\text{g/l}$: 0.017 $\mu\text{g/kg/day}$

Air: 0.001 $\mu\text{g/m}^3$: 0.0003 $\mu\text{g/kg/day}$
 0.01 $\mu\text{g/m}^3$: 0.003 $\mu\text{g/kg/day}$

Food: 0.005 $\mu\text{g/kg/day}$
 0.01 $\mu\text{g/kg/day}$

the number of individuals for which drinking water is the predominant source of exposure cannot be determined from the data since specific locations containing high concentrations of heptachlor epoxide in drinking water and low concentrations of heptachlor epoxide in ambient air and food are unknown. The relative source contribution data are based on estimated intake and do not account for a possible differential absorption rate for heptachlor epoxide by route of exposure. In addition, the effects of the chemical on the body may vary by different routes of exposure.

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IV. HUMAN EXPOSURE

Humans may be exposed to chemicals such as chlordane from a variety of sources, including drinking water, food, ambient air, occupational settings and consumer products. This analysis of human exposure to chlordane is limited to drinking water, food and ambient air because those media are considered to be sources common to all individuals. Even in limiting the analysis to these three sources, it must be recognized that individual exposure will vary widely based on many personal choices and on several factors over which there is little control. Where one lives, works and travels, what one eats, and physiologic characteristics related to age, sex and health status can all profoundly affect daily exposure and intake. Individuals living in the same neighborhood or even in the same household can experience vastly different exposure patterns.

Detailed information concerning the occurrence of and exposure to chlordane in the environment is presented in another document entitled "Occurrence of Pesticides in Drinking Water, Food and Air" (Johnston et al., 1984). This chapter summarizes the pertinent information presented in that document in order to assess the relative source contribution from drinking water, food and air.

In the Exposure Estimation section of this chapter, available information is presented on the range of human exposure and intake for chlordane from drinking water, food and ambient air for the 70 kg adult male. It is not possible to provide an estimate of the number of individuals experiencing specific combined exposures from those three sources. However, the

Summary section of this chapter provides some insight into the relative contributions of the three sources, especially drinking water, to the range of intake values suggested by the available data.

Exposure Estimation

Drinking Water. Levels of chlordane in drinking water vary from one location to another. Levels of chlordane monitored in the New Orleans Water Supply Study (U.S. EPA, 1975a) ranged from "none detected" to $<0.1 \mu\text{g}/\text{l}$, with a mean concentration of $<0.03 \mu\text{g}/\text{l}$. However, levels of chlordane in drinking water typically appear to be somewhat lower than these levels. For example, samples of finished drinking water were collected from 83 utilities in U.S. EPA Region V; only one of these finished drinking water samples contained chlordane at a level of $0.004 \mu\text{g}/\text{l}$ (U.S. EPA, 1975b). Chlordane may not be present in drinking water in some areas. The available monitoring data are not sufficient to determine regional variations in exposure levels for chlordane.

The daily intake of chlordane from drinking water was estimated using the assumptions presented in Table IV.B-1 and the values presented above. The estimates in Table IV.B-1 indicate that the daily intake of chlordane from drinking water ranges from 0.0 - $0.003 \mu\text{g}/\text{kg}/\text{day}$. However, the values presented do not account for variances in individual exposure or uncertainties in the assumptions used to estimate exposure.

Diet. Data are limited on the dietary intake of chlordane and oxychlordane, a metabolite of chlordane, in the United States. Daily food intake of oxychlordane for adults and for infants and toddlers by food group

TABLE IV.B-1

Estimated Intake of Chlordane from Drinking Water*

Drinking Water Concentration ($\mu\text{g/l}$)	Intake ($\mu\text{g/kg/day}$)
0.0	0.0
0.004	0.0001
0.03	0.0009
0.1	0.003

*Assumptions: 70 kg man consuming 2 l of water/day.

was estimated by FDA for fiscal year 1979 (FDA, 1982a,b) (Tables IV.B-2 and IV.B-3). The estimates of daily food intake are based on analyses of composite food samples obtained in market basket surveys. Intake calculated on the basis of weight ($\mu\text{g}/\text{kg}/\text{day}$) is greatest for the adult and lowest for the infant. For the adult male, infant and toddler, the highest intakes of oxychlordanes result from consumption of the meat, fish and poultry group.

Additional data were obtained on the estimated total dietary intake of chlordane and oxychlordanes for adults in the years 1974-1979 and infants and toddlers in the years 1975-1979 (FDA, 1980a,b, 1982a,b) (Table IV.B-4). The average total intakes of chlordane for adults, infants and toddlers over the years studied were 0.0009, 0.0010 and 0.0067 $\mu\text{g}/\text{kg}/\text{day}$, respectively. The average daily intakes of oxychlordanes for adults, infants and toddlers were 0.0023, 0.0016 and 0.0053 $\mu\text{g}/\text{kg}/\text{day}$, respectively.

Using the above data, the daily intakes of chlordane and oxychlordanes are estimated to be 0.0009 and 0.0023 $\mu\text{g}/\text{kg}/\text{day}$, respectively. These values do not account for variances in individual exposure.

It is expected that dietary levels of chlordane and oxychlordanes vary somewhat with geographical location, with higher levels of residues occurring in foods from areas near the sources of chlordane exposure. However, because of insufficient data, no estimates could be made of variations in intake by geographical region.

TABLE IV.B-2
Estimated Daily Intake of Oxychlordanes
by Food Class for Adult Male^{a,b}

Food Group	Intake (µg/kg/day) ^c
I. Dairy products	0.0010
II. Meat, fish and poultry	0.0019
III. Grains and cereals	0.0012
XI. Sugar and adjuncts	<u>0.00002</u>
Total	0.0042

^aSource: FDA, 1982a

^bNo intake values were obtained for chlordanes. Oxychlordanes is present as an alteration or byproduct of chlordanes.

^cObtained by dividing intake in µg/day by 69.1 kg for adult male; numbers do not add to total due to rounding.

TABLE IV.B-3

Estimated Daily Intake of Oxychlordan
by Food Class for 6-Month-Old Infant and 2-Year-Old Toddler^{a,b}

Food Group	Intake ($\mu\text{g}/\text{kg}/\text{day}$) ^c	
	Infant	Toddler
III. Other dairy and substitutes	--	0.0007
IV. Meat, fish and poultry	<u>0.0003</u>	<u>0.0024</u>
Total	0.0003	0.0031

^aSource: FDA, 1982b

^bNo intake values were obtained for chlordan. Oxychlordan is present as an alteration or byproduct of chlordan.

^cValues obtained by dividing intake in $\mu\text{g}/\text{day}$ by 8.2 kg for infants and 13.7 kg for toddlers.

TABLE IV.8-4

Estimated Total Daily Intake of Chlordane and Oxychlordane for Adult Male, Infant and Toddler^a

Year	Intake of Chlordane ($\mu\text{g/kg/day}$)			Intake of Oxychlordane ($\mu\text{g/kg/day}$)		
	Adult Male	Infant (6 months)	Toddler (2 years)	Adult Male	Infant (6 months)	Toddler (2 years)
1974	--	--	--	ND	--	--
1975	--	ND	ND	0.0006	Trace ^b	Trace
1976	0.0009	ND	0.01	0.0017	ND	Trace
1977	0.0011	ND	ND	0.0025	0.0011	0.0051
1978	0.0014	0.0051	0.0236	0.0025	0.0051	0.0078
1979	ND	ND	ND	0.0042	0.0003	0.0031
Average	0.0009	0.0010	0.0067	0.0023	0.0016	0.0053

^aSources: FDA, 1980a,b, 1982a,b^bTrace residues were not included in calculating the average; nondetected levels were treated as zero.

ND - Not detected

EPA has established a tolerance level of 300 $\mu\text{g/kg}$ for chlordane in and on the following raw agricultural commodities (40 CFR 180.122, July 1, 1981):

apples	cucumbers	peppers
apricots	dewberries	pineapples
beans	eggplant	plums (fresh prunes)
beet greens	grapes	potatoes
beets, with and without tops	kale	quinces
blackberries	kohlrabi	radish tops
blueberries (huckleberries)	lettuce	radishes, with and without tops
boysenberries	loganberries	raspberries
broccoli	melons	rutabagas
brussels sprouts	nectarines	squash
cabbage	okra	squash, summer
carrots	onions	strawberries
cauliflower	papayas	sweet potatoes
celery	peaches	tomatoes
cherries	peanuts	turnip greens
citrus fruits	pears	turnips, with and without tops
collards	peas	youngberries
corn		

Air. Levels of chlordane in the atmosphere vary from one location to another. The highest level of chlordane reported was 204 ng/m^3 (0.204 $\mu\text{g/m}^3$) (Kutz et al., 1976). Typical levels, however, are somewhat lower. An estimated mean level of chlordane in ambient air, based on information in Kutz et al. (1976), is 0.1 ng/m^3 (0.0001 $\mu\text{g/m}^3$). (However, this estimate is based on data from 1970-1972 and obtained in sampling locations having potentially high concentrations of pesticides in ambient air.) Additionally, concentrations of chlordane are below the limits of detection in some areas and may be as low as 0.0 ng/m^3 . The available monitoring data are not sufficient to determine regional variations in levels of exposure to chlordane.

The daily respiratory intake for chlordane from air was estimated using the assumptions presented in Table IV.8-5 and the values presented above.

TABLE IV.B-5

Estimated Respiratory Intake of Chlordane*

Air Concentration ($\mu\text{g}/\text{m}^3$)	Intake ($\mu\text{g}/\text{kg}/\text{day}$)
0.0	0.0
0.0001	0.00003
0.204	0.07

*Assumptions: 70 kg man inhaling 23 m³ of air/day (ICRP, 1975)

The estimates in Table IV.B-5 indicate that daily chlordane intake from air ranges from 0.0-0.07 $\mu\text{g}/\text{kg}/\text{day}$. The values do not account for variances in individual exposure or uncertainties in the assumptions used to estimate exposure. Data on levels of oxychlordane in ambient air were not sufficient for use in estimating respiratory intake of oxychlordane.

Summary

This section considers the relative contribution of drinking water, food and ambient air to the total human exposure to chlordane that could occur if a population was exposed to specific combinations of chlordane concentrations in drinking water, food and ambient air. Table IV.B-6 presents a general view of the total amount of chlordane that may be received by an adult male from air, food and drinking water. Three exposure levels in air, four exposure levels in drinking water and one exposure level from food are shown in the table.

The data in Table IV.B-6 have been selected from an infinite number of possible combinations of concentrations for the three sources. Whether exposure occurs at any specific combination of levels is not known; nor is it possible to determine the number of persons that would be exposed to chlordane at any of the combined exposure levels. The data represent possible exposures based on occurrence data and the estimated intake data.

A mean air level for chlordane of 0.0001 $\mu\text{g}/\text{m}^3$ was estimated. Assuming an air level of 0.0001 $\mu\text{g}/\text{m}^3$ and the estimated chlordane intake of 0.0009 $\mu\text{g}/\text{kg}/\text{day}$ from foods, drinking water would be the predominant source of exposure to chlordane at drinking water levels above 0.03 $\mu\text{g}/\text{l}$.

TABLE IV.B-6

Estimated Intake of Chlordane from the Environment by Adult Males

Concentration in Drinking Water ($\mu\text{g}/\text{l}$)	Estimated Total Intake in $\mu\text{g}/\text{kg}/\text{day}$ (% from drinking water) Based on a Concentration in Air ($\mu\text{g}/\text{m}^3$) of:		
	Low (0.0)	Intermediate (0.0001)	High (0.204)
Low (0.0)	0.0009 (0%)	0.0009 (0%)	0.07 (0%)
Intermediate (0.004)	0.001 (10%)	0.001 (10%)	0.07 (0.14%)
Intermediate (0.03)	0.002 (45%)	0.002 (45%)	0.07 (1.3%)
High (0.1)	0.004 (75%)	0.004 (75%)	0.07 (4.3%)

Intake from each source:

Drinking water: 0.0 $\mu\text{g}/\text{l}$: 0.0 $\mu\text{g}/\text{kg}/\text{day}$
 0.004 $\mu\text{g}/\text{l}$: 0.0001 $\mu\text{g}/\text{kg}/\text{day}$
 0.03 $\mu\text{g}/\text{l}$: 0.0009 $\mu\text{g}/\text{kg}/\text{day}$
 0.1 $\mu\text{g}/\text{l}$: 0.003 $\mu\text{g}/\text{kg}/\text{day}$

Air: 0.0 $\mu\text{g}/\text{m}^3$: 0.0 $\mu\text{g}/\text{kg}/\text{day}$
 0.0001 $\mu\text{g}/\text{m}^3$: 0.00003 $\mu\text{g}/\text{kg}/\text{day}$
 0.204 $\mu\text{g}/\text{m}^3$: 0.07 $\mu\text{g}/\text{kg}/\text{day}$

Food: 0.0009 $\mu\text{g}/\text{kg}/\text{day}$

The total estimated intake of chlordane is below 0.1 $\mu\text{g/kg/day}$. This value is far below the FAO/WHO acceptable daily intake of 1 $\mu\text{g/kg/day}$ (FDA, 1982a).

The relative source contribution data are based on estimated intake and do not account for a possible differential absorption rate for chlordane by route of exposure. The relative dose received may vary from the relative intake. In addition, the relative effects of chlordane on the body may vary by different routes of exposure.

Since no data were obtained on intake of oxychlordane from drinking water and ambient air, it is not possible to determine which of the three sources (drinking water, food, or air) is the major contributor to total intake of oxychlordane.

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V. HEALTH EFFECTS IN EXPERIMENTAL ANIMALS

General Toxicity

Effects of Acute Exposure. In the discussion of the health effects of acute exposure of animals to chlordane, heptachlor and heptachlor epoxide, durations of exposure from a single dose up to 15 daily doses will be considered acute.

Chlordane -- Values for the acute oral LD_{50} of chlordane in rats of various strains ranged from 83 mg/kg bw for pure cis-chlordane (Podowski et al., 1979) to 560 mg/kg bw for chlordane of unspecified purity (Ambrose et al., 1953), when delivered by gavage in lipophilic vehicle (Table V-1). The values determined for technical grade chlordane (see Chapter II.) by Gaines (1960) of 335 mg/kg bw (confidence limits 299-375 mg/kg bw) for male Sherman rats, and 430 mg/kg bw (391-473 mg/kg bw) for female rats of the same strain, are in good agreement with other values of 311 ± 46 mg/kg bw (Boyd and Taylor, 1969) and 350 ± 22 mg/kg bw (Gak et al., 1976) determined for technical grade chlordane in rats. A slightly lower value for rats (283 mg/kg bw) was reported by Ben-Dyke et al. (1970). Gak et al. (1976) found that mice of an unspecified strain were slightly less sensitive ($LD_{50} = 390 \pm 35$ mg/kg bw) and Golden hamsters were much less sensitive ($LD_{50} = 1720 \pm 135$ mg/kg bw) to chlordane than rats. Chlordane was not as toxic to rats when applied dermally (Gaines, 1960; Ben-Dyke et al., 1970). Harbison (1975) found that neonatal Sprague-Dawley rats were less sensitive than adult male rats to chlordane (analytical grade) intoxication when the insecticide was administered intraperitoneally. Pretreatment of the neonates with phenobarbital, however, reduced the LD_{50} from 1121 to 539 mg/kg bw, by enhancing the

TABLE V-1
Acute LD₅₀s for Chlordane

Species/ Strain	Sex/ Number	Weight/Age	Purity	Route	Vehicle	LD ₅₀ (mg/kg bw)	Comment	Reference
Rat/NR	NR/NR	NR	technical grade	oral	"innocuous solvent"	457	Tremors within 15 minutes, convulsions in 24 hours persisting until 48 hours. Deaths in 6 days.	Lehman, 1951
Rat/ Albino	NR/NR	NR	NR	oral	cottonseed oil	560	NC	Ambrose et al., 1953
Rat/ Sherman	M/70	175 g/90 days	technical grade	oral	peanut oil	335 (299-375)	Survival time: 48 hours-9 days for M and F; tremors, hyperexcitability, irritability and convulsions. Numbers in parentheses are confidence limits.	Gaines, 1960
Rat/ Wistar	F/70	200 g/90 days	technical grade	oral	peanut oil	430 (391-473)		
	M/141	50-60 g/ 56 days	technical grade	oral	cottonseed oil	311 ± 46	Death in 42 ± 18 hours. Clinical symptoms: stimulation of CNS irritability; phonation; piloerection; tremors and convulsions alternating with depression; hyporeflexia; lethargy; diarrhea; diuresis; anorexia; oligodipsia; hypothermia; low bw. At necropsy, local gastroenteritis, nephritis, hepatitis, adrenal and thymus stress reactions, vascular congestion, dehydration, loss of organ weight. LD ₅₀ = 237 mg/kg bw - estimated max. LD ₅₀ = 386 mg/kg bw - estimated max.	Boyd and Taylor, 1969
Rat/ Charles River	M/6-10/ group	150 g/6 weeks	cis- chlordane 99.9%	oral	corn oil	83	Deaths in 24 hours.	Podowski et al., 1979
Rat/NR	NR/NR	NR	NR	oral	olive oil	350 ± 22	NC	Gak et al., 1976
Mouse/NR	NR/NR	NR	NR	oral	olive oil	390 ± 35	NC	Gak et al., 1976
Hamster/ Golden	NR/NR	NR	NR	oral	olive oil	1720 ± 135	NC	Gak et al., 1976

TABLE V-1 (cont.)

Species/ Strain	Sex/ Number	Weight/Age	Purity	Route	Vehicle	LD ₅₀ (mg/kg bw)	Comment	Reference
Rat/NR	NR/NR	NR	commercial formulation	oral	NR	283	MC	Ben-Dyke et al., 1970
Rat/NR	NR/NR	NR	commercial formulation	dermal	NR	>600	MC	Ben-Dyke et al., 1970
Rat/ Sherman	M/70	175 g/90 days	technical grade	dermal	xylene	840 (750-941)	Survival time: 72 hours - 9 days	Gaines, 1960
	F/70	200 g/90 days	technical grade	dermal	xylene	590 (500-821)	96 hours - 25 days	
	F/70	200 g/90 days	technical grade	dermal	undiluted	530 (431-652)	120 hours - 15 days Numbers in parentheses are confidence limits.	
Rat/ Sprague- Dawley	M/F 10-20/ group	Neonates 2-5 days	analytical grade	1.p.	corn oil	1121 (904-1289)	Pretreatment with phenobarbital (40 mg/kg bw x 3) reduced the LD ₅₀ to 539 mg/kg bw (428-666). Numbers in parentheses are confidence limits.	Harbison, 1975
Rat/ Sprague- Dawley	M 10-20/ group	120-150 g/ adult	analytical grade	1.p.	corn oil	343 (258-451)	Numbers in parentheses are confidence limits.	Harbison, 1975
Rat/ Swiss- Webster	M/NR	20 g	cis- chlordane	1.p.	dimethyl sulfoxide	30	MC	Ivlie et al., 1972
			trans- chlordane	1.p.		130		

MC = No comment

NR = Not reported

1.p. = Intraperitoneal

ability of neonates to metabolize chlordane to its toxic intermediate. Pure cis-chlordane was more toxic than pure trans-chlordane to male Swiss-Webster mice when administered intraperitoneally (Ivie et al., 1972). The pure cis-isomer was also more toxic to rats than was technical grade chlordane. Further details of the LD₅₀ determinations are presented in Table V-1.

Symptoms of acute intoxication include CNS stimulation, as evidenced by irritability, tremors and convulsions (Boyd and Taylor, 1969; Stohlman et al., 1950; Hyde and Falkenberg, 1976). Boyd and Taylor (1969) described a wide range of CNS disturbances, including phonation; piloerection; tremors and convulsions alternating with lethargy; diarrhea; and food and water refusal. Necropsy of rats revealed vascular congestion, nephritis, hepatitis and decreased organ weight.

Other studies of acute exposure, other than LD₅₀ determinations, are described in Table V-2. Stohlman et al. (1950) have conducted an extensive study on dose response, vehicle effect and effect of route of administration of chlordane (described as "specially purified") on mortality of rats and rabbits. Chlordane was more toxic when administered orally to rats and rabbits in Tween-20 than in olive oil, as evidenced by greater incidence of mortality occurring at shorter periods following treatment. No deaths in rats occurred at a single oral dose of 100 mg/kg bw in Tween-20, although the same dose resulted in three deaths in eight rabbits within 2.5 hours. Multiple oral doses of 10 mg/kg bw/day in olive oil for 16 days resulted in 2 deaths among five rabbits. Increased mortality in rats after intraperitoneal administration and in rabbits after intravenous administration of chlordane was also observed (see Table V-2) (Stohlman et al., 1950).

TABLE V-2
Acute Toxicity of Chlordane

Species/ Strain	Sex/ Number	Weight/Age	Purity	Route	Vehicle	Dose	Duration	Effects	Reference
Rat/NR	NR/10 6 13 21 19	130-400 g	octa-chlor ("specially purified")	oral	Tween-20	0 mg/kg bw 100 mg/kg bw 200 mg/kg bw 300 mg/kg bw 1000 mg/kg bw	single dose	0/10 deaths 0/6 deaths 7/13 deaths (53%); tremors and convulsions. 13/21 deaths (62%); tremors and convulsions. 18/19 deaths (95%); tremors and convulsions.	Stohli et al., 1950
Rat/NR	NR/10 10 10	134-400 g	octa-chlor ("specially purified")	oral	olive oil	200 mg/kg bw 250 mg/kg bw 500 mg/kg bw	single dose	1/10 deaths (10%) in 30 days 6/10 deaths (60%) in 1-7 days 8/10 deaths (80%) in 2-5 days No mention of controls was made for this series.	Stohli et al., 1950
Rats/ Wistar V-5	N/A/ groups	200 g	cis- chlordane	oral	corn oil	0, 200 mg/kg bw	single dose	Fasting blood glucose increased to 147% of control; decreased liver glycogen (64%); increased serum urea (125%); increased activities of hepatic pyruvate carboxylase (183%), fructose- 1,6-diphosphatase (236%) and glucose-6-phosphatase (182%). Increased kidney cortical enzymes as above: 156%, 176%, 189% and 175%, respectively. Increased liver and kidney basal and fluoride-stimulated adeny- cyclase, and cyclic AMP. All the changes were significantly different from control levels (p<0.05).	Kacew and Singhal, 1973 Singhal and Kacew, 1976

TABLE V-2 (cont.)

Species/ Strain	Sex/ Number	Weight/Age	Purity	Route	Vehicle	Dose	Duration	Effects	Reference
Rabbit/NR	NR/6	1.6-3.3 kg	octa-klor ("specially purified")	oral	Tween-20	0 mg/kg bw	single dose	0/6 no effect	Stohman et al., 1950
	6				Tween-20	100 mg/kg bw		3/8 deaths (38%) 2-2.5 hours; dyspnea, tremors, convulsions.	
	13				Tween-20	200 mg/kg bw		5/6 (83%) deaths 2-4 hours, dyspnea, tremors, convulsions.	
	9				olive oil	300 mg/kg bw		5/13 (38%) deaths 6-22 days.	
	11				olive oil	400 mg/kg bw		7/9 (78%) deaths 4-31 days.	
Mouse/ CD-1					olive oil	600 mg/kg bw		8/11 (73%) deaths 2 hours to 8 days; dyspnea, tremors, convulsions.	Arnold et al., 1977
	7				olive oil	1000 mg/kg bw		7/7 (100%) deaths 3 hours to 4 days; dyspnea, tremors, convulsions.	
	M-F/NR	60-70 days	technical grade	oral	corn oil	100 mg/kg bw	single dose	No symptoms	
						300 mg/kg bw		Mild hypoactivity-no deaths	
						1000 mg/kg bw		100% mortality	
Mouse/ CD-1					corn oil	30 mg/kg bw	single dose	No symptoms	Arnold et al., 1977
						100 mg/kg bw		No symptoms	
						300 mg/kg bw		Intermittent tremors and ataxia followed by death.	
						1000 mg/kg bw		Intermittent tremors and ataxia followed by death.	
								100% mortality	
Rat/NR	M/12	50-60 g	octa-klor ("specially purified")	oral	diet	0.1%, 1000 mg/kg diet	10 days		Stohman et al., 1950
Rat/ Albino	NR/5	233-335 g	NR	oral	cottonseed oil	0, 6.25, 12.5, 25, 50, 100, 200 mg/kg bw/day	15 days	No observed symptoms of toxicity except for slight histologic changes in the liver of group exposed to <25 mg/kg/day. In the next highest group, 2 of the 5 animals died.	Ambrose et al., 1953a,b
Rat/ Wistar	M/ 6/group	80-130 g	technical grade	oral	diet	0, 5, 10, 20 or 50 mg/kg diet	14 days	Dose-related enzyme induction of liver aniline hydroxylase, aminopyrine diethylase and hexabarbitol oxidase, significant at 10-50 mg/kg diet, 10-50 mg/kg diet and 20-50 mg/kg diet, respectively (p<0.025). No-effect level = 5 mg/kg diet, lowest-effect level = 10 mg/kg diet.	Den Tonkelaar and Van Esch, 1974

TABLE V-2 (cont.)

Species/ Strain	Sex/ Number	Weight/Age	Purity	Route	Vehicle	Dose	Duration	Effects	Reference
Rabbit/NR	NR/5	1.6-3.3 kg	octa-klor ("specially purified")	oral	olive oil	5	0-15 days	0/5- no deaths until 53 days of feeding. 0/5- deaths at 14 days, 2 deaths at 16 days.	Stohlm et al., 1950
	5					10		4/4 deaths - 13 days	
	4					15		5/5 deaths - 10 days	
	5					20		14/14 deaths - 15 days	
	14					50		12/12 deaths - 9 days	
	12					100		6/6 deaths - 8 days	
	6					200			
						mg/kg bw/day			
Rat/NR	NR/NR	NR	cis- chlordane	NR	NR	200 mg/kg bw	single dose	Slight tremors, paralysis of hind legs, convulsions, hypothermia	Hrdina et al., 1974
Mouse/ Swiss	F/ 20/ group	NR	technical grade AEE reference standard	inhalation	air	current of air (18 ml/ sec) passed through 105 mm of chlor- dane in a satu- ration train	continuous for 14 days	No symptoms of toxicity for either grade of chlordane. Histologically, there was reticulation and oxyphilia of liver cytoplasm, congestion and cell proliferation of bronchiole lining.	Ingle, 1953
Rat/NR	NR/10	130-400 g	octa-klor ("specially purified")	i.p.	none	160 mg/kg bw 240 mg/kg bw 400 mg/kg bw	single dose	3/10 (30%) deaths in 9-10 days 8/10 (80%) deaths in 5-7 days 6/7 (86%) deaths in 2-6 days Pulmonary congestion and hemorrhaging.	Stohlm et al., 1950
	10				none				
	7				none				
	19				Tween-20	0 mg/kg bw	single dose	2/19 (11%) deaths in 7-9 days; adhesions and exudates.	
	10				Tween-20	50 mg/kg bw		1/10 (10%) deaths in 13 days	
	20				Tween-20	100 mg/kg bw		3/20 (15%) deaths in 1.5 hours- 6 days.	
	20				Tween-20	200 mg/kg bw		15/20 (75%) deaths in 40 minutes- 6 days. Tremors, convulsions.	
	10				olive oil	100 mg/kg bw	single dose	1/10 (10%) deaths in 8 days	
	10				olive oil	200 mg/kg bw		4/10 (40%) deaths in 2-3 days	
	10				olive oil	300 mg/kg bw		7/10 (70%) deaths in 3-6 days	

TABLE V-2 (cont.)

Species/ Strain	Sex/ Number	Weight/Age	Purity	Route	Vehicle	Dose	Duration	Effects	Reference
Rat/ Sprague- Dawley	M/2	255-275 g	technical grade	i.p.	3% Tween-80 isotonic saline	350 mg/kg bw	single dose	Deaths within 1 hour; mild tremors and disorientation in minutes; hypersensitivity progressing to clonic and recurrent ataxia, muscular rigidity, tetanic paralysis, hyperpnea; changes in EEG.	Hyde and Falkenburg, 1976
Mouse/ CD-1	M-F/MR	60-70 days	technical grade	i.p.	corn oil	100 mg/kg bw 300 mg/kg bw 1000 mg/kg bw	single dose	No symptoms of toxicity Mild hypoactivity, 50% mortality 100% mortality	Arnold et al., 1977
Mouse/ CD-1	M-F/MR	60-70 days	HCS-3260	i.p.	corn oil	30 mg/kg bw 100 mg/kg bw 300 mg/kg bw 1000 mg/kg bw	single dose	No symptoms of toxicity No symptoms of toxicity Intermittent tremors and ataxia among males. Intermittent tremors and ataxia among males, no mortality.	Arnold et al., 1977
Mouse/ Balb/ c Cr1	F/60/ group	neonate	cis- chlordane 100%	s.c.	sesame oil	0 0.075 mg/mouse	3 injections, 1 each on days 2, 3 and 4 of life	Significant differences from control: delayed eye opening ($p<0.001$); delayed vaginal opening ($p<0.001$), decreased bw at 4, 6 and 8 weeks.	Talamantes and Jung, 1977
						0.15 mg/mouse		Decreased bw at 4, 6 and 8 weeks, increased ovary weight ($p<0.05$).	
						0.075 mg/mouse	3 injections, 1 each on days 2, 3 and 4 of life	Significantly delayed eye opening ($p<0.01$), decreased bw at 4, 6, 8 and 10 weeks; decreased pituitary weight ($p<0.05$).	
						0.15 mg/mouse		Delayed vaginal opening ($p<0.05$), decreased bw at 4 weeks, decreased pituitary weight ($p<0.02$), increased ovary weight ($p<0.02$).	

TABLE V-2 (cont.)

Species/ Strain	Sex/ Number	Weight/Age	Purity	Route	Vehicle	Dose	Duration	Effects	Reference
Garbil/ Indian desert	M/20/ group	70-90 g/adult	technical grade	i.m.	petroleum ether	0, 25, 50, 75 mg/kg bw	single dose	Dose-related observed hyper- glycemia maximal 1 hour after treatment: blood glucose levels declined to control levels 1 week after treatment.	Saxena and Karel, 1976
Rabbit/HR	HR/15	1.6-3.5 kg	octa-klor ("specially purified")	i.v.	Tween-20 Tween-20 Tween-20 Tween-20 Tween-20	0 mg/kg bw 10 mg/kg bw 20 mg/kg bw 30 mg/kg bw 40 mg/kg bw	single dose	0/15 deaths 1/7 (14%) deaths in 14 hours 17/23 (74%) deaths in 7 minutes to 4 days. 10/10 (100%) deaths in 7 minutes to 2 days. 3/3 (100%) deaths in 15-30 minutes, dyspnea, tremors and convulsions.	Stohman et al., 1950

NR = Not reported

i.m. = intramuscular

i.p. = intraperitoneal

i.v. = intravenous

s.c. = subcutaneous

Ambrose et al. (1953) found no symptoms of toxicity except for intracytoplasmic bodies in the liver cells in all albino rats (groups of 5) treated orally for 15 days with 6.25 or 25 mg/kg bw/day chlordane in cottonseed oil. At doses of 50, 100 or 200 mg/kg bw/day, 2, 5 and 5 animals/group died. A single oral dose of 200 mg/kg bw cis-chlordane in corn oil resulted in significantly elevated liver and kidney gluconeogenic enzymes when compared with controls (Kacew and Singhal, 1973). Similar results were observed in gerbils treated intramuscularly with technical grade chlordane (Saxena and Karel, 1976).

Single oral doses of technical grade chlordane or of HCS-3260 (see Chapter III) to CD-1 mice resulted in no signs of toxicity at 30-100 mg/kg bw doses (Arnold et al., 1977). Mild hypoactivity was observed at 300 mg technical grade chlordane/kg bw and 100% mortality at a dose of 1000 mg/kg bw. Similar doses of HCS-3260 resulted in tremors, ataxia and death. Intraperitoneal doses of these preparations produced results similar to those observed after oral administration. Den Tonkelaar and Van Esch (1974) determined the dose response of technical grade chlordane administered in the diet for 14 days to groups of six male Wistar rats. Significantly elevated ($p < 0.025$) activities of aniline hydroxylase and aminopyrine demethylase occurred at a chlordane concentration of ≥ 10 mg/kg diet and of hexobarbital oxidase at ≥ 20 mg/kg diet. Enzyme activities at 5 mg/kg diet levels were not significantly different from control values. Continuous inhalations for 14 days of technical grade or AAEE reference standard chlordane (see Table V-2 for exposure) produced no outward signs of toxicity in Swiss mice; however, mild histological changes were seen in liver and lung tissues at necropsy (Ingle, 1953).

Neonatal female rats treated with subcutaneous injections of cis- or trans-chlordane (0.075 or 0.15 mg/mouse) on days 2, 3 and 4 of life had significantly delayed onset of eye opening and vaginal opening, decreased bw, decreased pituitary weight and increased ovary weight when compared with untreated controls (Talamantes and Jang, 1977). A dose-response relationship for these effects was not demonstrated for either isomer.

Heptachlor and Heptachlor Epoxide -- Acute oral LD_{50} values of heptachlor administered in lipophilic vehicles to rats, mice and hamsters are in good agreement (Table V-3). The range of values for rats was 71 g/kg bw for male Charles River rats (Podowski et al., 1979) to 162 mg/kg bw for female Sherman rats (Gaines, 1960). For male Sherman rats, the LD_{50} was 100 mg/kg bw. Gak et al. (1976) found that rats and hamsters were equally susceptible (LD_{50} = 105 ± 12 mg/kg bw and 100 ± 11 mg/kg bw, respectively). A value of 40 mg/kg bw in rats was reported for the less pure commercial formulation (Ben-Dyke et al., 1970). Heptachlor was slightly more toxic to mice (LD_{50} = 70 ± 7 mg/kg bw). Heptachlor applied dermally was less toxic than when administered orally to rats (Gaines, 1960; Ben-Dyke et al., 1970). Harbison (1975) found that upon intraperitoneal administration, analytical grade heptachlor was 7.5 times less toxic to neonatal Sprague-Dawley rats than to adult males. Pretreatment with phenobarbital, however, reduced the neonatal LD_{50} from 531 to 133 mg/kg bw by enhancing the neonates' ability to bioactivate the insecticide to the more toxic heptachlor epoxide. The acute oral LD_{50} of heptachlor epoxide in adult rats was ~60 mg/kg bw (NAS, 1977; Sperling and Ewinike, 1969; Podowski et al., 1979). The intraperitoneal LD_{50} of heptachlor epoxide in mice was 18 mg/kg bw (Ivie et al., 1972). Further details of the LD_{50} determinations are presented in Table V-3.

TABLE V-3
Acute LD50s for Heptachlor and Heptachlor Epoxide

Species/ Strain	Sex/ Number	Weight/Age	Compound and/or Purity	Route	Vehicle	LD50 (mg/kg bw)	Comment	Reference
Rat/NR	NR/NR	NR	"pure" heptachlor	oral	"innocuous solvent"	90	Tremors and convulsions, deaths occurred up to 6 days.	Lehman, 1951
Rat/ Sherman	M/60	175 g/90 days	technical heptachlor	oral	peanut oil	100 (74-135)	Tremors, hyperexcitability, irritability and convulsions Survival time: 6 hours - 7 days	Gaines, 1960
	F/90	200 g/90 days				162 (140-188)	Survival time: 5 hours - 11 days numbers in parentheses are confidence limits.	
Rat/NR	M/NR	adult	heptachlor purity NR	oral	NR	112	NC	Sperling and Ewinke, 1969
Rat/ Charles River	M 5-10/ group	150 g/6 weeks	heptachlor 99.9+%	oral	corn oil	71	Deaths occurred in 24 hours	Podowski et al., 1979
Rat/NR	NR/NR	NR	heptachlor purity NR	oral	olive oil	105 ± 12	NC	Gak et al., 1976
Mouse/NR	NR/NR	NR	heptachlor purity NR	oral	olive oil	70 ± 7	NC	Gak et al., 1976
Hamster/ Golden	NR/NR	NR	heptachlor purity NR	oral	olive oil	100 ± 11	NC	Gak et al., 1976
Rat/NR	NR/NR	NR	heptachlor commercial formulation	oral	NR	40	NC	Ben-Dyke et al., 1970
Rat/NR	NR/NR	NR	heptachlor commercial formulation	dermal	NR	195-250	NC	Ben-Dyke et al., 1970

TABLE V-3 (cont.)

Species/ Strain	Sex/ Number	Weight/Age	Compound and/or Purity	Route	Vehicle	LD50 (mg/kg bw)	Comment	Reference
Rat/ Sherman	M/100	175 g/90 days	technical heptachlor	dermal	xylene	195 (119-320)	Tremors, hyperexcitability, irritability and convulsions Survival time: 43 hours - 10 days	Gaines, 1960
	F/60	200 g/90 days				250 (200-313)	Tremors, hyperexcitability, irritability and convulsions Survival time: 96 hours - 13 days Numbers in parentheses are confidence limits.	
Rat/ Sprague- Dawley	M/F 10-20/ group	neonates 2-5 days	heptachlor analytical grade	1.p.	corn oil	531 (311-1020)	Pretreatment with phenobarbital (40 mg/kg bw x.3) 1.p. reduced LD50 to 133 mg/kg bw. Numbers in parentheses are confidence limits.	Harbison, 1975
Rat/ Sprague- Dawley	M/ 10-20/ group	120-150 g/ adult	heptachlor analytical grade	1.p.	corn oil	71 (41-103)	Numbers in parentheses are confidence limits.	Harbison, 1975
Rat/NR	M/NR	adult	heptachlor epoxide	oral	NR	62	NC	Sperling and Ewinika, 1969
Rat/ Charles River	M/ 6-10/ group	150 g/6 weeks	heptachlor epoxide	oral	corn oil	60	Deaths in 24 hours	Podowski et al., 1979
Rat/NR	M/NR	NR	heptachlor epoxide	oral	NR	46.5 61.3	NC	MAS, 1977
Mouse/ Swiss- Webster	F/NR							
	M/NR	20 g	reference heptachlor epoxide	1.p.	dimethyl sulfoxide	18	NC	Ivle et al., 1972

NC= No comment

NR= Not reported

1.p.= intraperitoneal

Other acute toxicity data for heptachlor and heptachlor epoxide are presented in Table V-4. Symptoms of intoxication include tremors, convulsions, paralysis and hypothermia (Hrdina et al., 1974; Yamaguchi et al., 1980). Oral doses of heptachlor have resulted in significantly elevated levels of serum GPT and serum aldolase coincident with histologically observed liver damage (Krampl, 1971). These effects were observed in female Wistar rats given a single oral dose of 98% pure heptachlor of 60 mg/kg bw or repeated oral doses of 7 or 12 mg/kg bw/day for 3-14 days. This study (Krampl, 1971) was actually conducted for 28 days. At 28 days, the serum levels of GPT and aldolase had returned to control levels, and damage to the liver was less severe. It is possible that the rats were developing a tolerance to heptachlor. At a high dose (200 mg/kg bw orally), which is above the oral LD₅₀, significantly increased ($p < 0.05$) activities of gluconeogenic enzymes were observed in male Wistar rats (Kacew and Singhal, 1973; Singhal and Kacew, 1976).

A single day or 5-7 days of dietary administration of heptachlor (96%) at a level of 10 mg/kg diet to rats resulted in alterations in liver function, as evidenced by changes in blood glucose, liver glycogen, GPT levels, acid and alkaline phosphatase activities, etc. (see Table V-4 for other parameters and directions of changes) (Enan et al., 1982). Single oral or intraperitoneal doses (30 or 100 mg/kg bw) of a heptachlor:heptachlor epoxide (27:75) preparation in CD-1 mice resulted in moderate to severe hypoactivity, ruffed fur and "partial mortality" (Arnold et al., 1977). At low dietary levels of >99% pure heptachlor (2-50 ppm or mg/kg diet) for 14 days, dose-related induction of hepatic aniline hydroxylase, aminopyrine demethylase and hexobarbital oxidase was observed in rats (Den Tonkelaar and Van Esch, 1974). Details of these studies are given in Table V-4.

TABLE V-4

Acute Toxicity of Heptachlor and Heptachlor Epoxide

Species/ Strain	Sex/ Number	Weight/ Age	Compound and/or Purity	Route	Vehicle	Dose mg/kg bw	Duration	Effects	Reference
Rat/ Wistar	F/24/ group	200- 300 g	heptachlor 98%	oral	vegetable oil	0, 60 mg/kg bw	single dose	Subgroups were sacrificed at 2, 24 and 72 hours. Significant changes ($p < 0.05-0.001$): elevated liver GPT and aldolase at 2 hours; decreased liver GPT and aldolase at 72 hours. Elevated serum GPT at 72 hours and serum aldolase at 24 and 72 hours. Changes coincided with slight to moderately severe histological liver damage.	Krampl, 1971
Rat/NR	NR/NR	NR	heptachlor	NR	NR	200 mg/kg bw	single dose	Slight tremors, hind leg paralysis, convulsions and hypothermia.	Hrdina et al., 1974
Rat/ Wistar	M/4/ group	200 g/ adult	heptachlor	oral	corn oil	0, 200 mg/kg bw	single dose	Increased fasting blood glucose level (748% of control); decreased concentration of liver glycogen in 1 hour (59%); increased hepatic pyruvate carboxylase (195%), phosphoenolpyruvate carboxykinase (200%), fructose-1,6-diphosphatase (239%) and glucose-6-phosphatase (158%); increased kidney cortical enzymes as above. 167%, 178%, 186% and 138%, respectively. Increased liver and kidney based and fluoride stimulated adenyl cyclase and cyclic AMP. All changes significantly different from control ($p < 0.05$).	Kacew and Singhal, 1973 Singhal and Kacew, 1976
Rat/NR	F/5	100- 150 g	heptachlor 96%	oral	diet	0 and 10 mg/kg diet	1 day of feeding	No effect on RBC count, significantly increased WBC count ($p < 0.05$), significantly elevated serum bilirubin ($p < 0.05$) decreased hepatic lipid content (n.s.), increased blood urea (n.s.) and increased serum cholesterol (n.s.), no change in blood glucose or hepatic protein content, decreased liver glycogen content (n.s.), decreased GPT (n.s.), increased GOT (n.s.), increased alkaline phosphatase ($p < 0.05$) and decreased acid phosphatase ($p < 0.05$) when compared with controls.	Enan et al., 1982

TABLE V-4 (cont.)

Species/ Strain	Sex/ Number	Weight/ Age	Compound and/or Purity	Route	Vehicle	Dose	Duration	Effects	Reference
Rat/NR	F/5	100- 150 g	heptachlor 96%	oral	diet	0 and 10 mg/kg diet	5 or 7 days* of feeding	No effects on RBC count, increased WBC and serum bilirubin ($p<0.05$), no change in hepatic lipid content; increased blood urea ($p<0.05$), no change in serum cholesterol, increased blood glucose ($p<0.05$), decreased liver glycogen content ($p<0.05$). Increased GPT (n.s.), decreased GOT (n.s.), increased acid and alkaline phosphatase ($p<0.05$), increased liver, heart and spleen weights (significance NR).	Enan et al., 1982
Rat/ Wistar	F/24/ group	200- 300 g	heptachlor 98%	oral	vegetable oil	0, 7 and 12 mg/kg bw/day	3-14 days	Subgroups of rats (6) were sacrificed at 3, 7 and 14 days. Significant changes ($p<0.05-0.001$): at 7 mg/kg day- elevated serum GPT and aldolase at 3, 7 and 14 days; reduced liver GPT and aldolase at 7 days, changes coincided with moderately severe liver damage; at 12 mg/kg/day - elevated serum GPT at 3, 7 and 14 days and aldolase at 7 and 14 days. Reduced liver GPT and aldolase at 7 and 14 days. Changes corresponded to moderately severe to severe histological liver damage.	Krampl, 1971
Rat/ Wistar	M/6/ group	80- 130 g	heptachlor >95%	oral	diet	0, 2, 5, 10, 20 or 50 mg/kg diet	14 days	Dose-related enzyme induction of liver aniline hydroxylase, aminopyrine demethylase and hexobarbital oxidase, significant at 2-50 mg/kg diet; 5-50 mg/kg diet and 5-50 mg/kg diet, respectively ($p<0.025$). No-effect level <2 mg/kg diet, lowest-effect level- 2 mg/kg diet.	Den Tonkelaar and Van Esch, 1974
Mouse/ CD-1	M-F/NR	60-70 days	heptachlor: heptachlor epoxide (25:75)	oral or i.p.	corn oil	30 mg/kg bw 100 mg/kg bw	single dose	Moderate hypoactivity and ruffed fur, 25% mortality. Severe hypoactivity and "partial mortality"	Arnold et al., 1977

TABLE V-4 (cont.)

Species/ Strain	Sex/ Number	Weight/ Age	Compound and/or Purity	Route	Vehicle	Dose	Duration	Effects	Reference
Rat/NR	NR/NR	NR	heptachlor epoxide	NR	NR	100 mg/kg bw	single dose	Slight tremors, hind leg paralysis, convulsions, hypothermia.	Hrdina et al., 1974
Rat/ Sprague- Dawley	M/2/ group	160- 200 g	heptachlor epoxide	1.p.	NR	0, 200 mg/kg bw	single dose	Violent convulsions 5 hours after treatment; animals were sacrificed - there was inhibition of brain synaptosomal Ca^{2+} - Mg^{2+} ATPase (see Chapter VII).	Yamaguchi et al., 1980

*The rats were maintained on the test diet for 5 days a week for 4 weeks. It was not clear whether rats were sacrificed after the 7th day of feeding, on the 9th day of the experiment or after the 5th day of feeding on the 7th day of the experiment.

NR- Not reported

1.p.- Intraperitoneal

n.s.- not significant

GPT- glutamate pyruvate transaminase

GOT- glutamate oxaloacetate transaminase

Effects of Subchronic and Chronic Exposures. The majority of the available studies on the effects of subchronic and chronic exposure to chlordane, heptachlor and heptachlor epoxide have been conducted by incorporating the insecticides into the diets of laboratory rats and mice. Many of the long-term studies were designed primarily to assess the carcinogenicity of these compounds; however, some data on toxicity were available.

Chlordane --

Subchronic Dietary Exposure. Table V-5 summarizes the studies discussed in this section.

A preliminary subchronic study was conducted to determine the dose levels of chlordane to be administered to Osborne-Mendel rats and B6C3F₁ mice for carcinogenicity testing (NCI, 1977a). Male and female rats, five of each sex per group, were administered diets containing chlordane (analytical grade 94.8%) in concentrations of 0-1600 ppm (1600 mg/kg diet) for 42 days. The rats were then maintained on chlordane-free diets for 2 weeks. The only criteria for toxicity were food consumption, body weight gain and mortality. No effects on body weight gain were observed at <400 mg/kg diet. Diets containing 800 mg/kg diet resulted in deaths of four females. A dietary level of 1600 mg/kg diet resulted in 100% mortality.

Groups of five male and five female mice received diets containing 0-320 ppm (320 ppm mg/kg diet) chlordane for 42 days, followed by 14 days observation (NCI, 1977a). No deaths occurred at <80 mg/kg diet. Diets of 160 mg/kg diet resulted in two deaths among males, none in females. All females

TABLE V-5
Effects in Rats and Mice of Subchronic and Chronic Dietary Exposure to Chlordane

Species/ Strain	Sex/Number	Weight/ Age	Purity	Dose (mg/kg diet)*	Duration	Effect	Reference
Rat/ Osborne- Mendel	M-F/5/sex/ group	NR	analytical grade chlordane, 94.8% (71.7% cis-, 23.1% trans- chlordane)	0, 50, 100, 200, 400, 800, 1600	42 days on test diet, 14 days of observation	No effects or bw gain at <400 mg/kg diet. At 800 mg/kg diet, 4/5 females died, no males died, but bw gain was reduced during first week only. At 1600 mg/kg diet, all died.	MCI, 1977a
Mouse/ B6C3F ₁	M-F/5/sex/ group	NR	analytical grade 94.8%	0, 20, 40, 80, 160, 320	42 days on test diet, 14 days of observation	No deaths at <80 mg/kg diet; at 160 mg/kg diet, 2/5 males, 0/5 females died. At 320 mg/kg diet 2/5 males, 5/5 females died.	MCI, 1977a
Rat/ Sprague- Dawley	M/42/group	91 days	technical grade	0 and 19.5 mg/kg bw/day based on food consumption data	90 days	No effect on food consumption when compared with controls. Decreased mean weekly bw gain compared with controls. No significant change in testicular and ventral prostate weight. Significant increased nuclear (642%) but not cytoplasmic androgen receptor site content of ventral prostate (p<0.05), decreased ventral prostate protein content (13% of control), RNA (35%) and DNA content (25%).	Shain et al., 1977
Rat/NR	M-F/NR	NR	technical grade or pure trans- chlordane	various dietary levels	91 days	Dose-related increased phosphorothioate detoxication. Increased activities of O-dimethylase and N-dimethylase over controls. The greatest increase was observed during the first week of treatment. Male rats were more sensitive than female rats. The NOEL for enzyme induction was 1 ppm (1 mg/kg diet).	Kinoshita and Kempf, 1970
Rat/NR	M-F/NR	NR	NR	0 and 1.2 mg/kg bw/day based on food	5 months	2 control rats died. No histopathological damage to lungs, heart, stomach, liver, kidney, spleen and testes. One treated rat had adenocarcinoma unrelated to treatment.	De Long and Ludwig, 1954

TABLE V-5 (cont.)

Species/ Strain	Sex/Number	Weight/ Age	Purity	Dose (mg/kg diet) ^a	Duration	Effect	Reference
Mouse/ C57BC 6N	M/200 controls Number of test mice NR	5 weeks	90% chlordane; 10% heptachlor	0	>36 weeks	0% "benign proliferative lesions" or carcinomas in liver.	Becker and Seil, 1979
				25		2% had benign proliferative lesions (clearly demonstrable foci, areas or nodular lesions which demonstrated neither microinvasion nor cytological atypia).	
				50		7% had benign proliferative lesions. It was unclear how many of the treated mice with benign proliferative lesions also had carcinomas or elevated α -fetoprotein levels. It was stated that of the 12 livers with benign proliferative lesions in the absence of carcinoma, none had elevated α -fetoprotein levels.	
Rat/NR	M/NR	NR	NR	0, 10, 20, 40, 80, 160, 320, 640 and 1280	400 days	Dose-related growth retardation; occasional liver pathology at 40 mg/kg; enlarged livers and liver pathology at ≥ 80 mg/kg; increased mortality at ≥ 640 mg/kg	Ambrose et al., 1953a,b
				0, 10, 20, 40, 80, 160, 320, 640 and 1280	400 days	Growth retardation at ≥ 320 mg/kg; enlarged livers and liver pathology at 80 mg/kg; increased mortality at ≥ 640 mg/kg	
Mouse/ CD-1	M-F/ 100/ sex/ group	6 weeks	analytical reference standard (technical grade)	0, 5, 25 and 50	18 months interim kill at 6 months of 10 mice/ sex/group	No effect on bw gain consumption was observed. There was a dose-related increased mean liver weight in treated males and females at 6 months and at 18 months. These increases were significant for all treated female groups, for the 50 mg/kg diet males at 6 months and for the 25 and 50 mg/kg diet males at 18 months. Survival was 14% for males and 24% for females at the highest dose-level and 51-73% for all other groups including controls, neoplastic lesions of the liver were observed (see Carcinogenicity Section). Hepatocytomegaly was observed in a dose-related increased incidence for all treated males and females. The incidence of hyperplastic nodules was dose-related at the 2 highest levels for males and females and significant ($p < 10^{-3}$). Re-evaluation of slides by other pathologists resulted in decreased incidences of nodules in favor of greater incidence of carcinomas (see Carcinogenicity Section).	Epstein, 1976 (review of IARC, 1973a)

TABLE V-5 (cont.)

Species/ Strain	Sex/Number	Weight/ Age	Purity	Dose (mg/kg diet)*	Duration	Effect	Reference
Rat/ Osborne- Mendel	N/10 matched, 60 pooled controls. 50 treated/ group	35 days	analytical grade, 94.8%	0	109 weeks	Mean bw of high-dose males and females were consistently decreased from controls. Most high-dose females had tremors at 44 weeks. All treated groups had clinical symptoms of toxicity throughout the study becoming progressively worse with time (loss of bw, rough and discolored hair, palpable masses and tumors). Among females, there was a significant (p=0.003) dose-related increase in mortality. No significant difference in mortality was observed for males. Histologic signs of aging were observed with equal frequency in control and treated rats. Neoplastic lesions were observed (see Carcinogenicity Section). Hyperplastic lesions were observed in thyroid glands of rats of all groups. These were follicular- and C-cell hyperplasia.	NCI, 1977a
				TMA 203.5, 407.0	80 weeks on test diet, 29 weeks ob- servation		
	F/10 matched, 60 pooled controls. 50 treated/ group	35 days		0	109 weeks		
				TMA 120.8, 241.5	80 weeks on test diet, 29 weeks ob- servation		
Mouse/ B6C3F ₁	N/20 matched, 100 pooled controls. 50 treated/ group	35 days	analytical grade 94.8%	0	91 weeks	No significant difference in bw gain between treated males and females and control males and females. High-dose males and females had tremors at 20 weeks. A few mice had general or local hair loss and a hunched appearance at 50 weeks. Abdominal distention, more prevalent in females, was observed in all groups. Significant differences in mortality were observed between low-dose and control males (p<0.02) and between high-dose and control males (p<0.01). No difference in mortality among females. Histological signs of aging were seen in all groups. Neoplastic lesions were observed (see Carcinogenicity Section). Hepatocytomegaly and hyperplastic lesions were observed infrequently in all groups.	NCI, 1977a
				TMA 29.9, 56.2	80 weeks on test diet, 10 weeks ob- servation		
	F/20 matched, 80 pooled controls. 50 treated/ group	35 days		0	91 weeks		
				TMA 30.1, 63.8	80 weeks on test diet, 10 weeks ob- servation		

TABLE V-5 (cont.)

Species/ Strain	Sex/Number	Weight/ Age	Purity	Dose (mg/kg diet) ^a	Duration	Effect	Reference
Rat/ Osborne- Mendel	M-F/20 of each sex/ group	50-55 g	technical	5	up to 2 years	No effect on food consumption, growth rate or mortality, no symptoms of toxicity, no gross or histopathological changes in liver, lungs, kidneys, pancreas, stomach, adrenals, thyroid, thymus, lymph, testes, ovaries, heart and spleen. Essentially the same as at 5 mg/kg diet; however, upon necropsy, minor liver damage (occasional hypertrophic cells, minor bile duct proliferation).	Ingle, 1952
				10			
				30		No effect on food consumption, growth rate, mortality or organ weights. Tremors could be induced after 80 weeks. Slight liver damage to a lesser degree than at 150 mg/kg diet. No kidney, lung, heart, adrenal, spleen or intestinal damage.	
				150		Slight reduction in growth rate after 60 weeks, greater in females, coinciding with periods of anorexia and tremors. Tremors after 26 weeks. Increased mortality at 48 weeks. Kidney and liver hypertrophy at 48 weeks in subgroup of 5 rats/sex. Upon histology duodenal ulcers, liver damage (centrilobular hypertrophy, nuclear karyorrhexis, karyolysis, necrosis, fatty degeneration, bile duct proliferation). Moderate to marked kidney, lung, myocardium, adrenal and spleen damage.	
				300		Reduced growth rate after 60 weeks, greater in females, coinciding with periods of anorexia and tremors. Hemorrhaging around eyes and nose associated with histological changes at 12 weeks. Increased mortality, 0 females and 1 male survived longer than 84 weeks. More severe liver, kidney, heart, adrenal, lung, myocardium and spleen damage.	
Dog/NR	NR/NR	NR	NR	0, 0.3, 3, 15 and 30	2 years	A review panel for WHO/FAO indicated that 3 mg/kg diet was a NOEL. The endpoints of the study were increased liver weight, and histopathological changes in the liver.	Mazeter, 1967

^aUnless otherwise stated

NR - Not reported

TMA - Time-weighted average

and two males died at the highest exposure level. Histological examinations were not performed or were not reported. Maximum tolerated doses were established at 400 and 800 mg/kg diet for female and male rats, respectively, and at 80 and 40 mg/kg diet for female and male mice, respectively.

Shain et al. (1977) found that feeding of technical grade chlordane to male Sprague-Dawley rats for 90 days at an average dose of 19.5 mg/kg bw/day had no effect on food consumption when compared with control-fed rats. Mean bw gain was significantly reduced in a subgroup of 12 randomly selected rats. In another subgroup of 24 rats, body weight was not reduced and no changes were observed in testicular or ventral prostate weights. Histological examination of the rats were not performed, since the study was designed to determine the effect of chlordane on prostate homeostasis. Rats were castrated 24 hours before sacrifice. Significant changes from controls were as follows: increased nuclear (642%) but not cytoplasmic androgen receptor site content, decreased RNA content (35%) and decreased DNA content (25%). Ventral prostate protein content was also reduced to 13% of control.

Induction of hepatic microsomal enzymes during 13 weeks of feeding various levels of chlordane or transchlordane to rats was reported by Kinoshita and Kempf (1970) in a meeting abstract. Dose-related increases in activities of enzymes involved in phosphorothioate detoxication, O-demethylase and N-demethylase activation, maximal during the first week of treatment, were noted. The no-effect level for enzyme induction was 1 ppm (1 mg/kg diet).

DeLong and Ludwig (1954) found that administration of an average daily dose of 1.2 mg technical grade chlordane/kg bw in the diet of male and female rats for 5 months resulted in no histopathologic damage to lungs, heart, stomach, liver, kidney, spleen or testes. The rats were fed dog pellets that had been treated by fog application of spray concentrations of chlordane. The average daily dose was calculated by the investigators from food consumption data. There was no mention of how many animals were tested; however, the investigators stated that two control rats died. One treated rat had a kidney adenocarcinoma, which was believed to be unrelated to chlordane treatment.

Chronic Dietary Exposure. Table V-5 summarizes the studies discussed in this section.

Ambrose et al. (1953a,b) conducted a study on the dietary effects of technical grade chlordane fed to groups of male and female rats for 400 days. The dietary levels used were 0, 10, 20, 40, 80, 160, 320, 640 and 1280 ppm (mg/kg) diet. Increased mortality was observed in the 640 and 1280 ppm groups and retarded growth was observed in all animals at ≥ 320 ppm. Some growth retardation was observed in male rats fed 20, 40 or 160 ppm chlordane diets, but no growth retardation was observed in female rats fed ≤ 160 ppm or in the male rats fed 10 or 80 ppm. Significantly enlarged livers and liver pathology were found in male and female rats fed chlordane at ≥ 80 ppm and liver pathology was occasionally found in male rats fed 40 ppm. Necrotic or degenerative pathology were present in the study animals.

Ingle (1952) described the dose-response effects in male and female Osborne-Mendel rats that had been exposed to chlordane in the diet for 2 years. High mortality among males and females, reduced growth rates, eye and nose hemorrhaging and severe histopathologic damage to liver, kidney, heart, adrenal, lung, myocardium and spleen were observed at 300 ppm (300 mg/kg diet). At a 150 mg/kg diet level, similar but less severe effects were seen. The effects at the 30 mg/kg diet level included inducible tremors and slight liver damage. No effects on food consumption, growth rate, mortality, organ weights or morphology were observed at this dose level. A diet containing 10 mg chlordane/kg produced minor liver damage, such as occasional hepatocytomegaly and mild bile duct hyperplasia. There was no effect on food consumption, growth rate or mortality at the 5 mg/kg diet levels. No symptoms of toxicity, gross or histopathologic changes in liver, kidney, lungs, pancreas, testes, ovaries, heart or spleen were noted.

Several studies that were designed to assess the carcinogenic potential of chlordane following long-term dietary exposure of rats and mice provide information on nontumor pathology. Disagreement among pathologists in the diagnosis and distinction of hepatic carcinoma from pre-cancerous lesions and hyperplasia in rodents has confounded the assessment of increased incidence of hyperplasia as a toxic rather than a carcinogenic effect.

Becker and Sell (1979), in an attempt to resolve these differences, studied elevated alpha-fetoprotein levels as an indicator of chlordane carcinogenicity in male C57B1/6N mice. The chlordane used was 90% chlordane and 10% heptachlor and was administered in the diet at concentrations of 0, 25 and 50 ppm (mg/kg diet) for at least 36 weeks to an unspecified number of

male mice. At 25 and 50 mg/kg diet, 2 and 7%, respectively, of the mice had benign proliferative lesions defined as "clearly demonstrable foci, areas or nodular lesions that demonstrated neither microinvasion nor cytological atypia." No lesions were observed in 200 control mice. Elevated levels of alpha-fetoprotein were observed in treated mice after 38 weeks and was always associated with primary hepatocellular carcinoma. The investigators stated that of 12 mice that had benign lesions without carcinoma, none had elevated alpha-fetoprotein levels. No growth was detected 3-7 months after six of the benign lesions were transplanted to compatible hosts, while 5-12 transplanted carcinomas had shown growth after 1.5-3 months. Thus, the benign lesions did not appear to be premalignant lesions.

An unpublished report by the International Research and Development Corporation (IRDC, 1973a) under contract to the Velsicol Chemical Corporation was reviewed by Epstein (1976). Groups of 100 male and 100 female CD-1 mice were administered technical grade chlordane in the diet at concentrations of 0, 5, 25 or 50 ppm (mg/kg diet) for 18 months. An interim kill of 10 mice/sex/group was performed at 6 months. No effect on body weight gain or food consumption was observed. At both 6 and 18 months, mean liver weights of all treated female groups were significantly increased in a dose-related manner. At 6 months, the 50 mg/kg diet level group of males had significantly increased mean liver weights when compared with controls. Survival, although underestimated because of the interim kill, was 14% for males and 24% for females in the 25 mg/kg diet group and 51-73% for all other groups, including controls. Increased incidence of hepatocytomegaly in all treated female and male groups occurred in a dose-related manner.

The incidence of hyperplastic nodules, as reported by IRDC (1973a), was dose-related at the two highest dose levels ($p < 1 \times 10^{-9}$). Re-evaluation of histologic slides by five of six other pathologists resulted in decreased incidences of the nodules in favor of greater incidences of carcinomas; however, total agreement was not obtained. Further discussion of this study is presented in the Carcinogenicity Section.

In the NCI (1977a) bioassay of the carcinogenicity of chlordane, groups of 50 male and 50 female Osborne-Mendel rats and groups of 50 of each sex B6C3F₁ mice were maintained on diets containing analytical grade chlordane (94.8%) for 80 weeks, followed by 29 and 10 weeks of observation for rats and mice, respectively. Controls for the rats consisted of 10 matched and 60 pooled untreated rats (controls from concurrent and recent bioassays of other related compounds) per sex. For male mice, 20 matched and 100 pooled controls were used, and for female mice, 20 matched and 80 pool controls were used. The doses were determined from the previously described preliminary subchronic study. Symptoms of toxicity and high mortality during the initial part of the study necessitated reductions in the dietary levels. A description of these adjustments, the dosing schedule and the resultant time-weighted average (TWA) dose, hereafter referred to as high doses and low doses, are presented in Tables V-6 and V-7 for rats and mice, respectively. For rats, mean body weight of high dose males and females were consistently depressed. High dose females had tremors at 44 weeks. All treated groups had individuals with symptoms of toxicity (loss of body weight, rough and discolored hair, palpable masses and tumors) that became progressively worse as the study continued. Among female rats there was a highly significant ($p=0.003$) dose-related increase in mortality. Mortality

TABLE V-6

Design Summary for NCI Bioassay of Chlordane in Osborne-Mendel Rats^a

Sex and Treatment Group	Initial No. of Animals ^b	Chlordane in Diet (ppm)	Time on Study		Time-Weighted Average Doses (ppm)
			Treated (weeks)	Untreated ^c (weeks)	
MALE					
Matched-Control	10	0	0	109	203.5
Low-Dose	50	400	33		
		100	14		
		50	33		
		0	0	29	
High-Dose	50	800	33		407.0
		200	14		
		100	33		
		0	0	29	
FEMALE					
Matched-Control	10	0	0	109	120.8
Low-Dose	50	200	33		
		100	14		
		50	33		
		0	0	29	

TABLE V-6 (cont.)

Sex and Treatment Group	Initial No. of Animals ^b	Chlordane in Diet (ppm)	Time on Study		Time-Weighted Average Doses (ppm)
			Treated (weeks)	Untreated ^c (weeks)	
High-Dose	50	400	33		241.5
		200	14		
		100	33		
		0	0	29	

^aSource: NCI, 1977a

^bAll animals were 35 days of age when placed on test.

^cWhen diets containing chlordane were discontinued, treated rats and their matched controls were fed plain feed diets (without corn oil) for 12 weeks and then control diets (2% corn oil added) for an additional 17 weeks.

^dTime-weighted average dose = $\frac{\Sigma(\text{dose in ppm} \times \text{no. of days at that dose})}{\Sigma(\text{no. of days receiving each dose})}$

TABLE V-7

Design Summary for NCI Bioassay of Chlordane in B6C3F₁ Mice^a

Sex and Treatment Group	Initial No. of Animals ^b	Chlordane in Diet (ppm)	Time on Study		Time-Weighted Average Doses (ppm)
			Treated (weeks)	Untreated ^c (weeks)	
MALE					
Matched-Control	20 ^e	0	0	91	29.9
Low-Dose	50	20	1	10	
		30	79		
High-Dose	50	0	0	10	56.2
		40	15		
		60	65		
FEMALE:					
Matched-Control	20 ^e	0	0	91	30.1
Low-Dose	50	40	1	10	
		30	79		
		0	0		

TABLE V-7 (cont.)

Sex and Treatment Group	Initial No. of Animals ^b	Chlordane in Diet (ppm)	Time on Study		Time-Weighted Average Doses (ppm)
			Treated (weeks)	Untreated ^c (weeks)	
High-Dose	50	80	15		63.8
		60	65		
		0	0	10	

^aSource: NCI, 1977a

^bAll animals were 35 days of age when placed on test.

^cWhen diets containing chlordane were discontinued, mice received the control diet (2% corn oil added) until termination.

^dTime-weighted average dose = $\frac{\sum(\text{dose in ppm} \times \text{no. of days at that dose})}{\sum(\text{no. of days receiving each dose})}$

^eInitially 10 animals of each sex were placed on test as matched controls; however, when the study was restarted, 10 additional animals of each sex were placed on test as matched controls.

was not significantly increased for male rats. Histopathologic signs of aging, such as chronic nephritis, biliary hyperplasia and chronic prostatitis, were observed with about equal frequency in all control and treated groups. The incidences of follicular and C-cell hyperplasia in thyroid glands of all treated and control groups were observed in a non-treatment-related incidence.

The results in mice indicated no difference in body weight gains among groups. Tremors were observed in high-dose males and females after 20 weeks. Mortality was significantly increased among treated males, but not females, in a dose-related manner. Histopathologic signs of aging commonly seen in mice occurred in all groups. Hepatocytomegaly and diffuse hepatic hyperplasia occurred infrequently in individuals of all groups.

An additional unpublished chronic study performed in dogs by Wazeter (1967) was reported by Vettorazzi (1975). The dogs were maintained for 2 years on diets containing chlordane at levels of 0, 0.3, 3, 15 or 30 ppm. The effects observed were increased liver weight and histologic changes in the liver. As reported by Vettorazzi (1975), a scientific review panel for WHO/FAO which examined this study and concluded that no effects were observed at exposures of 3 mg/kg diet or less. Other specific data on the experimental design or results of this study were unavailable.

Other Routes of Exposure. No data were available on inhalation or dermal exposure; however, several investigators (Karel and Saxena, 1976; Singhal and Kacew, 1976; Hyde and Falkenberg, 1976) have described effects following intraperitoneal and intramuscular injection of chlordane. Details of the studies are summarized in Table V-8. Karel and Saxena (1976) found

TABLE V-8
Effects of Subchronic Exposure to Chlordane by Other Routes

Species/ Strain	Sex/ Number	Weight/ Age	Purity	Route	Vehicle	Dose	Duration	Effects	References
Gerbil/ Indian desert	M/ 64 group	70-80 g/ adult	technical grade 60-75% pure	Intra- muscular	petroleum ether	0 and 2.5 mg/kg bw every 3 days	45 days	Increased blood glucose, serum protein, serum alkaline phos- phatase and serum acid phos- phatase levels of treated gerbils compared with controls.	Karel and Saxena, 1976
Rat/NR	NR/ 4/group	NR	cis- chlordane	Intra- peritoneal	corn oil	0 and 25 mg/kg bw/day	45 days	Significantly increased blood glucose, serum urea, decreased liver glycogen, increased kidney and liver pyruvate carboxylase, phosphoenolpyruvate carboxylkinase, fructose-1,6- diphosphatase and glucose-6- phosphatase ($p < 0.05$).	Singhal and Kacew, 1976
Rat/ Sprague- Dawley	M/ 4/group	255-275 g	technical grade	Intra- peritoneal	Tween-80- isotonic saline	0, 0.15, 1.75, and 25.0 mg/kg bw/day	48 days	Dose-related changes in EEGs increasing as exposure increased without symptoms of toxicity	Hyde and Falkenberg, 1976

NR = Not reported

impaired liver function in gerbils, as evidenced by increased blood glucose, serum protein, acid and alkaline phosphatase, after intramuscular injection with 15 doses of chlordane (60-75% pure) 2.5 mg/kg bw over 45 days. Similar results were observed for increased activities of gluconeogenic enzymes in rats following intraperitoneal injections of cis-chlordane at 25 mg/kg bw/day for 45 days (Singhal and Kacew, 1976). Dose-related changes in EEG without symptoms of toxicity were observed in rats treated intraperitoneally with <25.0 mg/kg bw/day for 45 days (Hyde and Falkenberg, 1976).

Heptachlor and Heptachlor Epoxide --

Dietary Exposure. Table V-9 summarizes the studies discussed in this section.

A preliminary subchronic study was conducted to determine the dose levels of heptachlor to be administered to Osborne-Mendel rats and B6C3F₁ mice for carcinogenicity testing (NCI, 1977b). Male and female rats, five of each sex per group, were administered diets containing technical grade heptachlor (~73% heptachlor, 22% transchlordane, 5% nonachlor) in concentrations of 0-320 ppm (320 mg/kg diet) for 42 days. The rats were then maintained on heptachlor-free diets for 2 weeks. The only criteria for toxicity were food consumption, body weight gain and mortality. No effects on body weight gain or food consumption were observed at <40 mg/kg diet. At 80 mg/kg diet, female rats had reduced body weight during the first week. Diets containing 160 mg/kg diet resulted in deaths of four females. A dietary level of 320 mg/kg diet resulted in the deaths of two male and five female rats.

TABLE V-9

Effects in Rats and Mice of Subchronic and Chronic Dietary Exposure to Heptachlor and Heptachlor Epoxide

Species/Strain	Sex/ Number	Weight/ Age	Compound and/or Purity	Dose (mg/kg diet) ^a	Duration	Effects	Reference
Rat/Osborne- Mendel	M and F/ 5/sex/ group	NR	Technical hepta- chlor (73% hepta- chlor, 22% trans- chlordane, 5% nonachlor	0 20 40 80 160 320	42 days on test diet, 14 days observation	No effect on bw gain or food con- sumption compared with controls. No effect on bw gain or food con- sumption compared with controls. Females had decreased bw gain during first week. 4/5 females died, no effect on bw gain or food consumption of males. 2/5 males died, 5/5 females died.	NCI, 1977b
Mouse/B6C3F ₁	M and F/ 5/sex/ group	NR	Technical hepta- chlor (73% hepta- chlor, 22% trans- chlordane, 5% nonachlor	0 20 40 80	42 days on test diet, 14 days observation	No effect on bw gain or food con- sumption. No effect on bw gain or food con- sumption. 5/5 males, 2/5 females died.	NCI, 1977b
Rat/Sprague- Dawley	M/ 42/group	91 days	99.8% heptachlor	0 and 1.29 mg/kg bw (based on food consumption data)	90 days	Moderate but significant ($p < 0.01$) reduction in food consumption com- pared with control rats. Decreased mean bw gain ($p < 0.01$). No signifi- cant change in testicular or ventral prostate weights. Significantly in- creased cytoplasmic, but not nuclear, androgen receptor site content of ventral prostate ($p < 0.05$). Decrease in available cytoplasmic androgen receptor site content ($0.05 < p < 0.01$). Decreased ventral prostate protein (13%) and cell loss (72%).	Shain et al., 1977
Rat/NR	M and F/ NR	NR	Heptachlor 99.1%	6 mg/kg bw ^b	52 weeks	Markedly decreased litter size, decreased lifespan of pups, development of cataracts.	Hestitzova, 1967

TABLE V-9 (cont.)

Species/Strain	Sex/ Number	Weight/ Age	Compound and/or Purity	Dose (mg/kg diet) ^a	Duration	Effects	Reference
Mouse/CD-1	M and F/ 100/sex/ group	7 weeks	Heptachlor:heptachlor epoxide (25:75)	0, 1.0, 5.0 and 10.0	18 months. Interim kill at 6 months of 10 mice/sex/group	females at 10 mg/kg diet had decreased, but not marked, by gain compared with controls, no differences in food consumption. There was a dose-related increase in mean liver weight in treated females and especially males at 6 and 18 months. These increases were significant for 5 and 10 mg/kg diet males and females at 18 months ($p < 0.01$) and for 1 mg/kg females at 18 months ($p < 0.05$). Survival was 29% for males and 30% for females at the 10 mg/kg diet level and 51-66% for all other groups. Neoplastic lesions of the liver were observed and are discussed in the Carcinogenicity Section. Hepatocytomegaly was observed in a dose-related increased incidence for all treated males and females. The incidence of hyperplastic nodules (described as an extension of the hepatocytomegaly) was dose-related at the 5 and 10 mg/kg diet levels for males and females and was significant ($p < 10^{-2}$). Re-evaluation of slides by other pathologists resulted in decreased incidences of the nodules in favor of greater incidences of carcinomas (see Carcinogenicity Section).	Epstein, 1976 (review of IROC, 1973b)

TABLE V-9 (cont.)

Species/Strain	Sex/ Number	Weight/ Age	Compound and/or Purity	Dose (mg/kg diet) ^a	Duration	Effects	Reference
Rat/Osborne- Mendel	M/10 matched, 60 pooled controls	35 days	Technical heptachlor (-75% heptachlor, 22% trans-chlordane, 5% nonachlor	0	111 weeks	Consistently decreased bw in both high-dose males and females compared with controls; a greater effect in males. The bw of low-dose groups were similar to controls. Both treated and control groups developed adverse clinical symptoms. In some females of both treated groups, vaginal bleeding developed after 80 weeks on test diet. Some low- dose females had mammary tumors. A dose-related, however not sig- nificant (p=0.17) increase in mortality was observed in males. A dose-related significant increase in mortality (p=0.04) was observed between treated female groups. Histologic signs of aging occurred with equal frequency in control and treated rats. Neo- plastic lesions were observed and are discussed in the Carcinogenicity Section. Hyperplastic lesions were observed in thyroid glands of rats of all groups. These were follicu- lar- and C-cell hyperplasias.	MCI, 1977b
	F/10 matched, 60 pooled controls	35 days		TMA 38.9, 77.9	80 weeks on test diet, 21 weeks observation		
				0	111 weeks		
Mouse/B6C3F ₁	M/20 matched, 100 pooled controls	35 days	Technical heptachlor (see above)	0	90-91 weeks	No appreciable differences in bw between treated and control mice were observed. Mice of all groups had sores and hair loss during first year. High dose females developed abdominal distention. Mice from all groups had rough coats, hair loss, sores and palpable masses. There was no apparent difference in male mortality among groups. There was a significant dose-related increase in mortality (p=0.02) between treated female groups. Hepatocytomegaly, diffuse hyperplasia and nodular hyperplasia were seen in livers of control and treated mice. Non- neoplastic lesions occurred in other organs randomly among all groups.	MCI, 1977b
				TMA 6.1, 13.8	80 weeks on test diet, 10 weeks observation		
				0	90-91 weeks		
	10/matched 80 pooled controls	35 days		TMA 9.0, 10.0	80 weeks on test diet, 10 weeks observation		

TABLE V-9 (cont.)

Species/Strain	Sex/ Number	Weight/ Age	Compound and/or Purity	Dose (mg/kg diet) ^a	Duration	Effects	Reference
Mouse/C3H	M/ 73 control 81 treated	3 weeks	Heptachlor purity NR	0 and 10	2 years	No hepatic vein or cardiac atrial thrombosis in controls. 13% of treated mice (10% of males and 15% of females) had venous occlusion with recent liver infarcts. Carcinomas and cirrhosis were also observed in some mice with thrombi. Of the mice with thrombi, 11% did not have carcinomas. A few mice with hepatic vein thrombosis had cardiac atrial thrombosis. Hyperplasia and hyperplastic nodules were observed in controls and treated mice.	Reuber, 1978 (histologic slides were from study of Davis, 1965)
Mouse/C3H	M and F/ 100/group	3 weeks	Heptachlor purity NR	0 and 10	2 years	Survival was 34% for controls, 30% for treated mice. "Hepatic hyperplasia and benign tumors" were seen in 2- to 3-fold greater frequency in treated mice. Histologic re-evaluation of slides by other pathologists resulted in decreased incidences of hyperplasias and nodules in favor of increased incidence of carcinoma (see the Carcinogenicity Section).	Epstein, 1976 (review of Davis, 1965)
Rat/CF	M/20/group	10 weeks	Heptachlor purity NR	0 1.5 3.0 5.0 7.0 10.0	110 weeks	Mortality Loss of bw in treated males, most predominant in 10 mg/kg diet group. Increased liver weights in treated males especially in highest dose group. Reduced food consumption in 10 mg/kg diet group. Liver lesions described as "Chlorinated hydrocarbon" type seen in 30% of 7.0 mg/kg diet group, 17% in 10 mg/kg diet group and 0% in other groups.	Epstein, 1976 (review of Witherup et al., 1955)

TABLE V-9 (cont.)

Species/Strain	Sex/ Number	Weight/ Age	Compound and/or Purity	Dose (mg/kg diet) ^a	Duration	Effects	Reference
Rat/CF (cont.)	F/20/group	10 weeks	Heptachlor purity NR	0	110 weeks	Mortality Loss of bw, increased liver weight, not seen in females. "Chlorinated hydrocarbon" type liver lesions seen in 17% of 7.0 mg/kg diet group. 50% of 10 mg/kg diet group and 0% in other groups. The incidence of malignant tumors is discussed in the Carcinogenicity Section.	
				1.5			
				3.0			
				5.0			
				7.0			
				10.0		55%	
Rat/CD	F/ 54 control 25 treated/ group	NR	99.9% heptachlor: 96% heptachlor epoxide (75:25)	0	2 years	Age related lesions seen in all groups. Spontaneous lesions and tumors seen in all groups (multiple cell type hypertrophy and telangiectasia in anterior pituitary and adrenal hypertrophy). Hepatocytomegaly exceeded control levels at 7.5, 10.0 and 12.5 mg/kg diet levels.	Epstein, 1976 (review of Jolley et al., 1966)
				5			
				7.5			
				10.0			
				12.5			
Mouse/CD	M/ 79 treated F/ 81 treated	3 weeks	Heptachlor epoxide purity NR	0 and 10 ppm	2 years	No hepatic vein or cardiac atrium thrombi, no cirrhosis of liver in controls. 10% of treated mice (7% of males, 11% of females) developed hepatic vein thrombosis; 9% (5 males, 9 females) treated mice had venous occlusion. Carcinomas and cirrhosis were observed in some mice with thrombi. Of the mice with thrombi, 11% did not have carcinomas. A few had cardiac atrium thrombosis as well. 12/78 treated males, 15/81 treated females had cirrhosis.	Reuber, 1977a, 1978 (histologic slides obtained from study of Davis, 1965)

TABLE V-9 (cont.)

Species/Strain	Sex/ Number	Weight/ Age	Compound and/or Purity	Dose (mg/kg diet) ^a	Duration	Effects	Reference
Mouse/C3H	M and F/ 100/group	NR	Heptachlor epoxide purity NR	0 and 10	2 years	Survival was 34% for controls, 9.5% for treated mice. Premature death was high. "Hepatic hyperplasia and benign tumors" were seen in 2-fold greater frequency in treated mice. Histologic re-evaluation of the slides by other pathologists resulted in decreased incidences of hyperplasias and nodules in favor of increased incidences of carcinomas (see Carcinogenicity Section).	Epstein, 1976 (review of Doris, 1965)
Rat/CFW	M/25/group	7 weeks	Heptachlor epoxide purity NR	0 0.5 2.5 5.0 7.5 10.0	110 weeks	<u>Mortality</u> No difference in food consumption or growth rate. Hepatic cell vacuolization in all treated groups. Hepatocytomegaly, degeneration and regeneration in unspecifed groups. Tumor data are discussed in the Carcinogenicity Section. 24% 44% 36% 28% 52% 48%	Epstein, 1976 (review of Mithrup et al., 1959)
	F/25/group	7 weeks		0 0.5 2.5 5.0 7.5 10.0	110 weeks	No food consumption differences, no differences in growth rate. Dose-related increase in liver weights. Hepatic cell vacuolization in all treated groups. Hepatocytomegaly, degeneration and regeneration in unspecifed groups. Tumor data are discussed in the Carcinogenicity Section. 24% 44% 36% 28% 52% 48%	

^amg/kg diet unless otherwise stated

^bIt is unclear from the study whether or not this is a daily dose and administered by feeding. The author stated that heptachlor was administered with "food" and "the applied dose was 6 mg/kg bw".

NR = Not reported; TWA = Time-weighted average

Groups of five male and five female mice received diets containing 0-80 ppm (0-80 mg/kg diet) heptachlor for 42 days followed by 14 days observation (NCI, 1977b). No deaths and no effects on body weight gain or food consumption occurred at <40 mg/kg diet. At 80 mg/kg diet, all male and two female mice died. Histological examinations of the rats or the mice were not performed or were not reported. Maximum tolerated doses were established at 160 and 80 mg/kg diet for male and female rats, respectively, and 40 mg/kg diet for male and female mice.

Shain et al. (1977) found that feeding of heptachlor (99.8%) to male Sprague-Dawley rats for 90 days at an average dose of 1.29 mg/kg bw/day significantly reduced the food consumption and mean body weight gain in a subgroup of 12 randomly selected rats. In another subgroup of 22 rats, body weight was not reduced and no changes were observed in testicular or ventral prostate. Histological examination of the rats were not performed, since the study was designed to determine the effect of heptachlor on prostate homeostasis. Rats were castrated 24 hours before sacrifice. Cytoplasmic, but not nuclear, androgen receptor site content of the ventral prostate was significantly increased. Ventral prostate protein content was reduced to 13% of control levels and cell number was reduced to 18% of control.

Induction of hepatic microsomal enzymes during 13 weeks of feeding various dietary levels of heptachlor or heptachlor epoxide to rats was reported by Kinoshita and Kempf (1970) in a meeting abstract. There were dose-related increases in the activities of enzymes involved in phosphorothioate detoxication and activities of O-demethylase and N-demethylase, maximal during the first week of treatment. The no-effect level for enzyme induction was reported to be 1 ppm (1 mg/kg diet).

Mestitzova (1967) found that heptachlor (98.1%) administered with food at a dose of 6 mg/kg bw for 52 weeks resulted in markedly decreased litter sizes, decreased lifespan of pups and the development of cataracts in parent rats and pups. It is unclear whether the 6 mg/kg bw dose was administered daily or whether it represented a total dose consumed over 1 year. In the former case, the dose would be quite high, roughly corresponding to a concentration of 120 mg/kg diet, assuming that a rat consumes 5% daily of its body weight as food. In the latter case, the dose would be quite low, resulting in a daily average of ~0.016 mg/kg bw/day.

As with chlordane, several long-term feeding studies with heptachlor or heptachlor epoxide were designed as carcinogenicity studies but provide toxicologic data as well, and there has been disagreement among pathologists on the distinction between malignant and benign lesions of the liver induced by heptachlor or its epoxide.

In the unpublished report by IRDC (1973b) reviewed by Epstein (1976), a 25:75 mixture of heptachlor:heptachlor epoxide was administered to groups of 100 male and 100 female CD-1 mice in the diet at 0, T.O, 5.0 and 10.0 ppm (mg/kg diet) for 18 months. An interim kill of 10 mice/sex/group was performed at 6 months. Female mice at the 10 mg/kg diet level had decreased body weight gain; otherwise, no other effect on body weight or food consumption was observed. Mean liver weight increased significantly in a dose-related manner in treated females and males at 6 and 18 months. This increase was more marked in males. Survival, although underestimated due to the interim kill, was 29% for males and 30% for females at the highest dose level and 51-66% for all other groups, including controls. Increased

incidence of hepatocytomegaly in all treated female and male groups occurred in a dose-related manner. The incidence of hyperplastic nodules, as reported by IRDC (1973a), was dose-related at the two highest levels for males and females and was highly significant ($p < 1 \times 10^{-9}$). Re-evaluation of histologic slides by six other pathologists resulted in several different interpretations; however, the overall result was a decreased incidence of hyperplasia in favor of a greater incidence of carcinoma. Further discussion of this study is presented in the Carcinogenicity Section.

Epstein (1976) reviewed the results of several other unpublished reports: 1) Davis (1965), an FDA report on heptachlor and heptachlor epoxide; 2) Witherup et al. (1955), a study by the Kettering Laboratories on heptachlor; 3) Jolley et al. (1966), a study by Kettering on a heptachlor and heptachlor epoxide mixture; and 4) Witherup et al. (1959), a Kettering study on heptachlor epoxide. Discussion of these studies with respect to toxic effects follows. Further discussion of the carcinogenic effects is presented in the Carcinogenicity Section.

Davis (1965) administered 0 and 10 ppm (10 mg/kg diet) heptachlor or heptachlor epoxide to groups of 100 male and 100 female C3H mice for 2 years. For heptachlor, low survival was observed for treated (30%) as well as control (34%) mice. For heptachlor epoxide, survival was 9.5%. A 2-fold increase over controls in the incidence of hepatic hyperplasia and benign tumors was observed for both compounds, although re-evaluation of the slides by four other pathologists changed the interpretation in favor of a greater number of hepatomas.

Witherup et al. (1955) studied the effects of heptachlor on groups of 20 male and 20 female CF rats. The insecticide was administered in dietary concentrations of 0-10 ppm (10 mg/kg diet). Mortality among test groups was not dose-related (see Table V-9). Loss of body weight, decreased food consumption and increased liver weight were seen among treated males, but not females, and were most marked at 10 mg/kg diet. Liver lesions described as "chlorinated hydrocarbon" type and considered to be non-neoplastic were observed in 17% of males and 50% of females at 10 mg/kg diet, and 38% of males and 17% of females at 7 mg/kg diet. At dose levels at or below 5.0 mg/kg diet, none of these lesions was observed. This study was not re-evaluated by other pathologists.

Jolley et al. (1966) administered a 75:25 mixture of heptachlor: heptachlor epoxide to groups of 25 female CD rats in the diet at concentrations 5-12.5 ppm (12.5 mg/kg diet) for 2 years. A group of 54 rats received diets free of insecticide. Mortality was increased in a somewhat dose-related way (see Table V-9). Comprehensive histological evaluations were performed. Spontaneous lesions and tumors were present in all groups and included multiple cell type hypertrophy, telangiectasia in the anterior pituitary and adrenal hypertrophy. The incidence of hepatocytomegaly was increased over control levels at 7.5, 10.0 and 12.5 mg/kg diet. This study was not re-evaluated by other pathologists.

Witherup et al. (1959) studied the effect of heptachlor epoxide in groups of 25 male and 25 female CFN rats for 110 weeks. The dietary concentrations ranged from 0.5-10.0 ppm (mg/kg diet). Control rats received heptachlor epoxide-free diets. No differences were observed with respect to

food consumption or growth rate. There was a dose-related increase in liver weight in females. Hepatic cell vacuolization occurred in treated males. Hepatocytomegaly, degeneration and regeneration in unspecified groups were reported. Mortality was higher than control level for all treated groups, but a dose-response relationship was not observed. This study was not re-evaluated by other pathologists.

Reuber (1977a, 1978), using slides from the study of Davis (1965), found hepatic vein thrombosis and cirrhosis among the heptachlor and heptachlor epoxide (10 mg/kg diet) treated mice. These conditions were not observed in any of the 127 control slides available for review. For heptachlor-treated mice, 13% (10% of males, 15% of females) had hepatic vein thrombosis and 6% had venous occlusion with recent liver infarcts. Thrombosis of the cardiac atrium was also present in some mice with hepatic vein thrombosis. The incidence of cirrhosis was 2/86 treated males and 5/77 treated females. For heptachlor epoxide, 10% of treated mice (7% of males, 11% of females) had hepatic vein thrombosis and 9% had venous occlusion. Cardiac atrium thrombosis was present in some mice. The incidence of cirrhosis was 12/78 treated males and 15/81 treated females. In addition, liver carcinomas were also observed in the treated mice.

In the NCI (1977b) bioassay of the carcinogenicity of heptachlor, groups of 50 male and 50 female Osborne-Mendel rats and groups of 50 of each sex B6C3F₁ mice were maintained on diets containing technical grade heptachlor for 80 weeks plus 21 and 10 weeks of observation for rats and mice, respectively. Controls for the rats consisted of 10 matched and 60 pooled untreated rats (controls from concurrent and recent bioassays of other related

compounds) per sex. For male mice, 20 matched and 100 pooled controls were used, and for female mice, 10 matched and 80 pooled controls were used. The doses were determined from the previously described preliminary subchronic study. Changes in dose levels were necessitated by developing symptoms of toxicity. A description of these adjustments, the dosing schedule and the resultant TWA doses, hereafter referred to as high doses and low doses, are presented in Tables V-10 and V-11 for rats and mice, respectively.

For rats, mean body weight of high-dose males and females were consistently depressed especially in males. The low-dose groups had growth rates similar to controls. Adverse clinical signs such as loss of body weight, rough and discolored hair and palpable masses, developed in treated and untreated groups. In some females from both dose groups, vaginal bleeding developed after 80 weeks. A dose-related, but not significant, increase in mortality was observed for treated males. The increase was significant between treated female groups. Histopathologic signs of aging, such as chronic nephritis, biliary hyperplasia and chronic prostatitis, were observed with about equal frequency in all control and treated groups. The incidence of follicular- and C-cell hyperplasia in thyroid glands of treated and control groups were observed to be non-treatment-related.

The results in mice indicated no differences in body weight gains among groups. Sores and hair loss occurred in treated and control mice during the first year. Abdominal distention and hair loss was prevalent in high-dose females. Adverse clinical signs developed in mice of all groups. Male mortality was unaffected; however, there was a significant dose-related increase in mortality between treated female groups. Hepatocytomegaly and diffuse hyperplasia occurred infrequently in individuals of all groups.

TABLE V-10

Design Summary for NCI Bioassay of Heptachlor in Osborne-Mendel Rats^a

Sex and Treatment Group	Initial No. of Animals ^b	Heptachlor in Diet (ppm)	Time on Study		Time-Weighted Average Doses (ppm)
			Treated (weeks)	Untreated ^c (weeks)	
MALE					
Matched-Control	10	0	0	111	38.9
Low-Dose	50	80	31		
		20	14		
		10	35		
		0	0	30	
High-Dose	50	160	31		77.9
		40	14		
		20	35		
		0	0	30	
FEMALE					
Matched-Control	10	0	0	111	25.7
Low-Dose	50	40	22		
		60	9		
		20	14		
		10	35		
		0	0	30	

TABLE V-10 (cont.)

Sex and Treatment Group	Initial No. of Animals ^b	Heptachlor in Diet (ppm)	Time on Study		Time-Weighted Average Doses (ppm)
			Treated (weeks)	Untreated ^c (weeks)	
High-dose		80	22		51.3
		120	9		
		40	14		
		20	35		
		0	0	30	

^aSource: MCI, 1977b

^bAll animals were 35 days of age when placed on test.

^cWhen diets containing heptachlor were discontinued, treated male rats and their matched controls were fed plain feed diets (without corn oil) for 11 weeks, then control diets (2% corn oil added) for an additional 18 weeks; treated female rats and their matched controls were fed plain feed diets for 9.5 weeks, then control diets for an additional 20 weeks.

Time-weighted average dose = $\frac{\sum(\text{dose in ppm} \times \text{no. of days at that dose})}{\sum(\text{no. of days receiving each dose})}$

TABLE V-11

Design Summary for MCI Bioassay of Heptachlor in B6C3F₁ Mice^a

Sex and Treatment Group	Initial No. of Animals ^b	Heptachlor in Diet (ppm)	Time on Study		Time-Weighted Average Doses (ppm)
			Treated (weeks)	Untreated ^c (weeks)	
MALE					
Matched Control	20 ^e	0	0	90-91	6.1
Low-Dose	50	10	18	10	
		5	62		
High-Dose	50	0	0	10	13.8
		20	17		
		10	50		
FEMALE					
Matched-Control	10	0	0	90-91	9.0
Low-Dose	50	20	17	10	
		10	13		
		5	50		
		0	0		

TABLE V-11 (cont.)

Sex and Treatment Group	Initial No. of Animals ^b	Heptachlor in Diet (ppm)	Time on Study		Time-Weighted Average Doses ^d (ppm)
			Treated (weeks)	Untreated ^c (weeks)	
High-Dose	50	40	17		18.0
		20	13		
		10	50		
		0	0	10	

^aSource: MCI, 1977b

^bAll animals were 35 days of age when placed on test.

^cWhen diets containing heptachlor were discontinued, mice received control diet (2% corn oil added) until termination.

^dTime-weighted average dose = $\frac{\sum(\text{dose in ppm} \times \text{no. of days at that dose})}{\sum(\text{no. of days receiving each dose})}$

^eInitially 10 males were placed on test as matched controls.

Other Routes of Exposure. No data were available on inhalation or dermal exposure; however, several investigators (Kacew et al., 1973; Singhal and Kacew, 1976; Hrdina et al., 1974) have described effects following intraperitoneal and intramuscular injections of heptachlor and heptachlor epoxide. Details of the studies are summarized in Table V-12. Kacew et al. (1973) administered heptachlor or heptachlor epoxide to Wistar rats intramuscularly for 45 days. The doses were 0, 3 or 15 mg/kg bw/day for heptachlor and 0, 1 or 5 mg/kg bw/day for heptachlor epoxide. For both compounds, liver weights were decreased in all treated rats when compared with controls. Dose-related increases in enzymes of gluconeogenic metabolism in livers and kidneys were significant. Similar results were obtained following intraperitoneal injections of heptachlor (Singhal and Kacew, 1976). Hrdina et al. (1974) administered 0, 3 or 15 mg/kg bw/day heptachlor or heptachlor epoxide by an unspecified route to rats for 45 days and observed significantly decreased cerebro-cortical acetyl-choline levels.

Target Organ Toxicity

Chlordane.

Effects on the Liver -- Histopathologic changes in the liver were observed in rats at dietary levels of chlordane >18 mg/k diet for males and females (Ambrose et al., 1953a,b). At exposures as low as 10 mg/kg diet, female rats had slight increases in liver weight. Increased incidences of hepatocytomegaly, hepatic hyperplasia and nodules were reported in a carcinogenicity study (IRDC, 1973a); however, re-examination of slides indicated that the nodules were hepatomas (Epstein, 1976). Chlordane feeding induced benign proliferative lesions in the liver of a strain of mice that historically and concurrently did not develop lesions when untreated

TABLE V-12
Effects of Subchronic Exposure to Heptachlor and Heptachlor Epoxide by Other Routes

Species/ Strain	Sex/ Number	Weight/ Age	Compound and/or Purity	Route	Vehicle	Dose (mg/kg bw day)	Duration	Effects	Reference
Rat/ Wistar	M/F/ group	100 g	heptachlor purity NR	1.m.	corn oil	0, 3 or 15	45 days	No significant effect on bw, kidney, adrenal, thymus or heart weights at either dose level. Significantly decreased liver weights at both dose levels ($p<0.05$) and significantly decreased testes weight at 15 mg/kg bw/day ($p<0.05$) when compared with controls. Dose-related levels significantly increased over control levels of kidney and liver pyruvate carboxylase, phosphoenolpyruvate carboxylase, fructose-1,6-diphosphatase and glucose-6-phosphatase ($p<0.05$). Dose-related, significantly elevated levels of blood glucose and serum urea and decreased liver glycogen ($p<0.05$).	Kacev et al., 1973
Rat/NR	NR/ 4/group	NR	heptachlor purity NR	1.p.	corn oil	0 and 15	45 days	Significantly increased blood glucose, serum urea, liver and kidney levels of pyruvate carboxylase, phosphoenolpyruvate carboxylase, fructose-1,6-diphosphatase and glucose-6-phosphatase; significantly decreased liver glycogen compared with controls ($p<0.05$).	Singhal and Kacev, 1976
Rat/NR	NR/NR	NR	heptachlor purity NR	NR	NR	0, 3 and 15	45 days	Significantly decreased cerebro-cortical acetylcholine; no change in brain-stem norepinephrine; significantly decreased brain stem 5-hydroxytryptophan.	Hrdina et al., 1974

TABLE V-12 (cont.)

Species/ Strain	Sex/ Number	Weight/ Age	Compound and/or Purity	Route	Vehicle	Dose (mg/kg bw day)	Duration	Effects	Reference
Rat/ Wistar	M/F/ group	100 g	heptachlor epoxide purity NR	i.m.	corn oil	0, 1 or 5	45 days	No significant effect on bw, kidney, adrenal, thymus testes or heart weights at either dose level. Significantly decreased liver weights at both dose levels. Dose-related levels significantly increased over control levels of liver and kidney pyruvate carboxylase, phosphoenpyruvate carboxylase, fructose-1,6-diphosphatase and glucose-6-phosphatase ($p < 0.05$). Dose-related, significantly elevated levels of blood glucose and serum urea; decreased liver glycogen ($p < 0.05$).	Kacew et al., 1973
Rat/NR	NR/NR	NR	heptachlor epoxide purity NR	NR	NR	0, 1 or 5	45 days	Significantly decreased cerebrocortical acetylcholine level, no change in brain stem norepinephrine levels.	Hrdina et al., 1974

NR= Not reported

i.m.= intramuscular

i.p.= intraperitoneal

(Becker and Sell, 1979). The incidence of hepatocytomegaly and hyperplasia was not increased over control incidence in B6C3F₁ mice (NCI, 1977a). Dose-related, increasingly severe liver damage was observed by Ingle (1952) in rats treated chronically with dietary levels of chlordane. The damage included centrilobular hypertrophy, nuclear karyorrhexis, karyolysis, necrosis, fatty degeneration and bile duct proliferation. Reticulation and oxyphilia of liver cytoplasm was observed after acute inhalation exposure (Ingle, 1953).

Several investigators noted changes in liver function, as evidenced by enzyme activity changes. Kinoshita and Kempf (1970) found that the activities of the microsomal enzymes, such as phosphorothioate detoxicating enzyme, O-demethylase and N-demethylase, were significantly increased in rats following technical grade chlordane or transchlordane feeding. Induction of liver aniline hydroxylase, aminopyrine demethylase and hexobarbital oxidase in rats was observed by Den Tonkelaar and Van Esch (1974). Enhancement of gluconeogenesis was described in rats, as evidenced by increased blood glucose, increased serum urea, decreased liver glycogen, increased activities of hepatic pyruvate carboxylase, phosphoenolpyruvate carboxykinase, fructose-1,6-diphosphatase and glucose-6-phosphatase (Singhal and Kacew, 1976; Kacew and Singhal, 1973).

Effects on Endocrine System and Reproductive Organs -- Hyperplastic lesions, such as follicular-cell and C-cell hyperplasia of the thyroid, were observed in rats treated with 120-407 mg/kg bw of chlordane (NCI, 1977a). Shain et al. (1977) found changes in ventral prostate homeostasis of rats at dose levels of ~20 mg/kg bw, as evidenced by increased nuclear androgen

receptor site content and decreased protein, RNA and DNA content. Delayed vaginal opening, increased ovary weight and decreased pituitary weights were seen in neonatal mice treated with 0.075 mg/mouse (Talamantes and Jang, 1977).

Effects on CNS -- Clinical and behavioral manifestations of chlordane poisoning have been noted in rats and mice. Tremors, hyperexcitability, irritability, phonation, piloerection and convulsions were commonly observed after acute exposures (Lehman, 1951; Gaines, 1960; Boyd and Taylor, 1969; Stohlman et al., 1950; Arnold et al., 1977; Hrdina et al., 1974; Hyde and Falkenberg, 1976) and chronic exposures (Ingle, 1952; NCI, 1977a). Hyde and Falkenberg found dose-related (between 0.15 and 25 mg/kg bw/day) changes in EEGs of rats given daily intraperitoneal injections of chlordane.

Effects on Other Organs -- Moderate to marked histopathologic damage to kidneys, lungs, myocardia, adrenals and spleen in rats fed chlordane at 150 mg/kg diet became more severe at 300 mg/kg diet (Ingle, 1952). Duodenal ulcers and hemorrhaging around eyes and nose were also observed. Congestion and proliferation of bronchiole lining cells were seen in lungs of mice after acute inhalation exposure to chlordane (Ingle, 1953). Increased activities of kidney cortical pyruvate carboxylase, phosphoenolpyruvate carboxykinase, fructose-1,6-diphosphatase and glucose-6-phosphatase were observed in rats after acute oral exposure to chlordane (Kacew and Singhal, 1973).

Heptachlor and Heptachlor Epoxide.

Effects on the Liver -- Moderate to severe histopathologic changes in the liver were accompanied by elevated serum GPT and serum aldolase levels in rats after acute or subchronic oral exposures to heptachlor up to 14 days. At 28 days, however, the serum enzyme levels returned to control levels, and liver damage was less severe (Krampl, 1971). Similar findings by Enan et al. (1982) also included elevated serum bilirubin and serum cholesterol, decreased hepatic lipid content, decreased liver glycogen, increased serum glutamate-oxalacetate transaminase, increased alkaline phosphatase and decreased acid phosphatase levels after oral exposure of rats to heptachlor. Increased incidences of hepatocytomegaly, diffuse hepatic hyperplasia and hyperplastic nodules were common findings in mouse livers after chronic feeding of heptachlor and heptachlor epoxide (IRDC, 1973b; NCI, 1977b; Davis, 1965; Witherup et al., 1955, 1959; Jolley et al., 1966); however, re-evaluation of slides from the IRDC (1973b) study and of the Davis (1965) study, indicated that the hyperplastic conditions had been overdiagnosed by the original investigators and that the conditions of hepatocellular carcinoma had been underdiagnosed (Epstein, 1976). Cirrhosis and hepatic vein thrombosis were observed by Reuber (1977a) in mice treated with heptachlor or heptachlor epoxide.

Several investigators noted changes in liver function, evidenced by changes in enzyme activities. The microsomal enzymes of phosphorothioate detoxication, O-demethylase and N-demethylase activities increased in rat livers after dietary exposure to heptachlor or heptachlor epoxide (Kinoshita and Kempf, 1970). Induction of liver aniline hydroxylase, aminopyrine demethylase and hexobarbital oxidase occurred in rats after oral exposure to

heptachlor (Den Tonkelaar and Van Esch, 1974). Decreased liver weights were accompanied by enhanced gluconeogenesis in rats following intramuscular or intraperitoneal injections of heptachlor or heptachlor epoxide (Kacew et al., 1973; Singhal and Kacew, 1976). Increased liver pyruvate carboxylase, phosphoenolpyruvate carboxykinase, fructose-1,6-diphosphatase and glucose-6-phosphatase activities; increased blood glucose and serum urea; and decreased liver glycogen were evidence of enhanced gluconeogenesis.

Effects on Endocrine System and Reproductive Organs -- Increased but not significantly dose-related incidence of hyperplastic lesions of the thyroid, including follicular-cell and C-cell hyperplasia, were observed in rats following chronic dietary heptachlor exposure (NCI, 1977b). Increased cytoplasmic nuclear androgen receptor site content of the ventral prostate, decreased prostate protein content and cell loss were indicative of changes in ventral prostate homeostasis in rats exposed to heptachlor (Shain et al., 1977). Kacew et al. (1973) observed decreased testes weights in rats following intramuscular injections of heptachlor. Testes weight was not increased following heptachlor epoxide injection. Vaginal bleeding developed in female rats after chronic dietary exposure to heptachlor (NCI, 1977b); however, the incidence was low and not significantly different from controls.

Effects on CNS -- Clinical and behavioral manifestations of heptachlor and heptachlor epoxide poisoning were observed in rats and mice. Tremors, hyperexcitability, irritability, convulsions, hind leg paralysis, hypoactivity and hypothermia were common findings after acute exposures to heptachlor and heptachlor epoxide (Lehman, 1951; Gaines, 1960; Hrdina et al.,

1974; Arnold et al., 1977; Yamaguchi et al., 1980). Hrdina et al. (1974) found decreased levels of cerebrocortical acetylcholine and brain stem 5-hydroxy-tryptophan associated with tremors and convulsions.

Effects on Other Organs -- In addition to the hepatic vein thrombosis observed in mice following heptachlor or heptachlor epoxide feeding, a few cases of thrombosis of cardiac atrium were observed (Reuber, 1977a). In the kidney cortex, enzymes associated with gluconeogenesis have increased activities in rats after exposures to heptachlor or heptachlor epoxide (Kacew et al., 1973). Mestitzova (1967) reported that rats fed heptachlor developed cataracts of the eye.

Other Effects

Studies on the carcinogenicity, mutagenicity, teratogenicity and reproductive effects of chlordane, heptachlor and heptachlor epoxide exposure in laboratory animals are discussed in this section.

Carcinogenicity. Many of the reports (Davis, 1965; IRDC, 1973a,b; Witherup et al., 1955, 1959; Jolley et al., 1966) on the carcinogenicity of these chemicals have not been published; however, Epstein (1976) has reviewed the results of these studies in a published article that was based on a Statement of Suspension Testimony at the Environmental Protection Agency Hearing on Heptachlor/Chlordane, Washington, DC, August 26, 1975. Epstein (1976) also presented results of independent statistical reanalyses and histological re-evaluations of the results of these studies. Among the pathologists who re-examined the histological slides was Melvin D. Reuber, M.D., who published an article (Reuber, 1977b) on his re-evaluation of the

Davis (1965) report. Reuber (1978) also presented his re-evaluations of Davis (1965), IRDC (1973a,b) and NCI (1977a,b); however, the tumor incidence data was reported as total numbers from combined studies, and as such, are of little value for comparison purposes.

In addition to the information provided by Epstein (1976), whose paper should be consulted for further details, the results of the National Cancer Institute bioassays on chlordane (NCI, 1977a) and heptachlor (NCI, 1977b), a study by Cabral et al. (1972) and a study by Becker and Sell (1979) are presented in the following discussion.

Chlordane -- In the IRDC (1973a) study, analytical grade chlordane was administered in the diet to groups of 100 male and 100 female CD-1 mice at concentrations of 5, 25 or 50 ppm (mg/kg diet) for 18 months. The mice were 6 weeks of age at the beginning of the experiment. Controls consisted of similar groups maintained on chlordane-free diets. Data on survival and signs of toxicity were presented in the Effects of Subchronic and Chronic Exposures Section. A highly significant ($p < 1 \times 10^{-5}$) dose-related increase in the incidence of nodular hyperplasia in the liver of males and females of the 25 and 50 mg/kg diet groups was noted. The incidence of hepatomas, however, was not significantly different from the incidence among controls and followed no dose-related trend. Epstein (1976) reported the results of a histological re-examination of IRDC slides performed by Reuber, who found very high incidences of hepatic carcinoma rather than hyperplastic nodules that, at the 25 and 50 mg/kg diet levels, were highly significant ($p < 1 \times 10^{-5}$). Three other independent pathologists have concurred with Reuber (Epstein, 1976). The incidences of hepatic carcinoma, as determined by Reuber, are presented in Table V-13.

TABLE V-13

**Incidence of Hepatocellular Carcinoma in Mice
Following Chronic Dietary Administration of Chlordane**

Dose (mg/kg diet)	No. of Tumor Bearing Mice/No. of Mice Examined (% Positive)	
	Male	Female
Strain CD-1^a		
0	3/33 (9%)	0/45 (0%)
5	5/55 (9%)	0/61 (0%)
25	41/52 (79%)	32/50 (64%)
50	32/39 (82%)	26/37 (70%)
Strain B6C3F₁^b		
0 (pooled)	17/92 (18%)	3/78 (4%)
0 (matched)	2/18 (11%)	0/19 (0%)
29.9	16/48 (33%)	NA
30.1	NA	3/47 (6%)
56.2	43/49 (88%)	NA
63.8	NA	34/49 (69%)

^aIRDC, 1973a; study reviewed by Epstein (1976), tumor incidence as determined by Reuber. Tumor incidences significantly different from controls for 25 and 50 mg/kg diet ($p < 1 \times 10^{-6}$).

^bNCI (1977a); Dose-related trend significant at $p < 0.0001$.

NA = Not applicable

The NCI (1977a) bioassay was conducted in groups of 50 male and 50 female B6C3F₁ mice and Osborne-Mendel rats. Technical grade chlordane (94.8%) was administered in the diet for 80 weeks, followed by a 10-week observation period for mice and a 29-week period for rats. TWA high and low-dose concentrations were 56.2 and 29.9 ppm (mg/kg diet), respectively, for male mice and 63.8 and 20.1 mg/kg diet, respectively, for female mice. For rats, the TWA high-dose and low-dose concentrations were 407 and 203.5 mg/kg diet, respectively, for males and 241.5 and 120.8 mg/kg diet, respectively, for females. The designs of the dose schedules were presented previously in Tables V-6 and V-7. For the mouse studies, controls consisted of 20 matched controls for each sex and 100 and 80 pooled controls for males and females, respectively. For the rat study, 10 rats of each sex served as matched controls and 60 rats of each sex served as pooled controls. Comprehensive histological examination was performed on major organs and gross lesions of all animals that died spontaneously, were killed when moribund, or were killed at the termination of the study. Histological examination was precluded in some cases because of cannibalism or autolysis. Mortality data and symptoms of toxicity were discussed in the Effects of Subchronic and Chronic Exposures Section. Male mice had the following incidences of hepatocellular carcinoma: 43/49 (88%) high-dose, 16/48 (33%) low-dose, 2/18 (11%) matched controls and 17/92 (20%) pooled controls. The dose-related trend was highly significant ($p < 0.0001$). Female mice also had a highly significant dose-related increase ($p < 0.0001$) in the incidence of hepatocellular carcinomas as follows: 34/49 (69%) high-dose, 3/47 (6%) low-dose, 0/19 (0%) matched controls and 3/78 (4%) pooled controls. These results are compared with the IRDC results in Table V-13. Miscellaneous neoplasms, such as alveolar-cell carcinoma and adenoma in lung, malignant lymphoma of

hematopoietic system, thyroid follicular-cell adenoma, tubular adenoma of kidney, mammary gland fibroadenoma and neurofibroma and lacrimal gland adenomas, were observed randomly and infrequently among all groups. In rats, only two hepatocellular carcinomas were observed, one in a low-dose male and one among the pooled controls. Follicular-cell carcinomas of the thyroid were observed with greater frequency and were found to be dose-related in combination with C-cell carcinomas. Comparison of the incidences of these tumors in males or females with pooled or matched controls resulted in some significant dose-related trends that were inconsistent and ambiguous. A highly significant dose-related increase in the incidence of fibrous histiocytoma ($p=0.0007$) was observed for male rats. These were not observed in females. The investigators did not believe these lesions to be related to chlordane treatment, since these tumor types had occurred spontaneously with variable frequency throughout the bioassay program. Tumors of the pituitary were observed relatively frequently among treated and control rats, and no significant trends were found. Miscellaneous neoplasms occurred randomly in treated and control groups. The incidences of the tumors that showed significant trends, albeit not considered treatment-related, are presented in Table V-14.

An unspecified number of male C57B1/6N mice, a strain that historically did not develop spontaneous liver tumors, were administered a 90:10 mixture of chlordane:heptachlor at dietary concentrations of 25 or 50 ppm (mg/kg diet) (Becker and Sell, 1979). Controls consisted of 200 mice that did not develop any liver lesions over 18 months of observation. Animals dying during the test were necropsied, as were three mice in each group per week beginning at 6 months. Primary hepatocellular carcinoma developed in 16

TABLE V-14

Incidence of Thyroid Carcinomas and Adenomas, Fibrous Histiocytoma and Pituitary Carcinoma and Adenoma Following Chronic Dietary Administration of Chlordane to Osborne-Mendel Rats^{a,b}

	Male Rats ^c				Female Rats ^c			
	Pooled Control	Matched Control	Low Dose	High Dose	Pooled Control	Matched Control	Low Dose	High Dose
<u>Thyroid</u>								
Follicular-cell carcinoma	1/51 (2)	0/6 (0)	1/34 (3)	2/31 (6)	1/58 (2)	0/10 (0)	2/43 (5)	2/32 (6)
Follicular-cell adenoma	3/51 (6)	0/6 (0)	0/34 (0)	4/31 (13)	2/58 (3)	0/10 (0)	2/43 (5)	4/32 (13)
C-cell carcinoma	1/51 (2)	0/6 (0)	1/34 (3)	2/31 (6)	5/58 (9)	3/10 (30)	0/43 (0)	3/32 (9)
C-cell adenoma	3/51 (6)	0/6 (0)	4/34 (12)	3/31 (10)	7/58 (12)	0/10 (0)	3/43 (7)	7/32 (22)
<u>Malignant fibrous Histiocytoma (any site)</u>								
	2/58 (3)	0/8 (0)	1/44 (2)	7/44 (16)	1/60 (2)	1/10 (10)	1/49 (2)	0/44 (0)
<u>Pituitary</u>								
Carcinoma	2/48 (4)	0/6 (0)	1/34 (3)	1/34 (3)	0/52 (0)	0/10 (0)	1/46 (2)	0/37 (0)
Adenoma	14/48 (29)	0/6 (0)	9/34 (26)	7/34 (21)	23/52 (44)	6/10 (60)	7/46 (15)	1/37 (30)

^aSource: MCI, 1977a

^bIncidence expressed as no. of tumor-bearing rats/no. of tissues examined.

^cThe numbers in parentheses are the incidences expressed as percents.

treated mice (27% of surviving mice). The investigators did not relate tumor incidence to dose. Transplantation of cells of 12 carcinomas to recipient mice resulted in five growing tumors that were histologically similar to the primary hepatocellular carcinoma. Chlordane was found to be a more potent carcinogen than acetylaminofluorene, administered to C57B1/6N mice at 0.045 or 0.03% of the diet (450 or 300 mg/kg diet). Carcinomas appeared in seven acetylaminofluorene-treated mice (18% of survivors) after 61 weeks. Of six acetylaminofluorene-induced tumors transplanted, three showed growth at 2.4-4 months. Thus, chlordane was demonstrated to induce hepatocellular carcinomas in a strain of mice that does not spontaneously develop liver lesions.

Heptachlor and Heptachlor Epoxide -- As mentioned in the introduction to this section, Epstein (1976) has reviewed a number of unpublished reports on the carcinogenic effects of heptachlor and heptachlor epoxide. The U.S. EPA (1980a) has compiled summary tables of the review of Epstein (1976), which, with some modifications, are presented in Table V-15. More detailed discussions of these reports are supplied in the text that follows.

Witherup et al. (1955) administered heptachlor to groups of 20 male and 20 female CF rats at dietary levels of 1.5-10.0 ppm (mg/kg diet). Similar groups served as controls. The rats were 10 weeks of age and were maintained on the test diet for 110 weeks, at which time survivors were sacrificed for necropsy. Data on mortality and signs of toxicity were discussed previously in the Effects of Subchronic and Chronic Exposures Section. Benign and malignant tumors were randomly distributed among test and control groups, with greater incidences observed for females, especially at

TABLE V-15

Summary of Unpublished Carcinogenicity Data in Rats and Mice*

Authors	Species/ Strain	Formulation Heptachlor (H): Epoxide (HE): Chlordane (C)	Concentration mg/kg diet		Conclusions of Original Authors	Carcinogenicity	
			H	HE		Independent Histological Re-evaluation	Comments of Epstein, 1976
Witherup et al., 1955	rat/CF	H of unspecified purity	1.5, 3.0, 5.0, 7.0, 10.0	-	Tumor incidence "proportionately" distributed in all tests and control groups.	Not undertaken	1. Test diets pre- pared crudely and poorly documented. 2. Author's data demonstrate statisti- cally significant increase in malig- nant and any tumors in multiple sites in some female test groups.
Witherup et al., 1959	rat/CFM	HE of unspecified purity	-	0, 0.5, 2.5, 5.0, 7.5, 10.0	Tumor incidence "unrelated" to HE content in diets. Excess hepatomas in test animals is acknowledged, but discounted. Also unusual malignant tumors in males and females	Hepatocarcino- genic and multiple site malignant tumors	1. Test diets pre- pared crudely and study poorly documented. 2. Kettering data statistically significant, for incidence of total tumor-bearing animals and for liver and pitu- itary tumors. 3. Histological re-evaluation showed hepato- carcinomas. 4. Hepatocarcino- genicity statisti- cally signif- icant.

TABLE V-15 (cont.)

Authors	Species/ Strain	Formulation Heptachlor (H); Epoxide (HE); Chlordane (C)	Concentration mg/kg diet		Conclusions of Original Authors	Carcinogenicity	
			H	HE		Independent Histological Re-evaluation	Comments of Epstein, 1976
Jolley et al., 1966	rat/CD	Mixture of 25% HE (99.9% pure) and 75% H (96.0% pure)	5.0, 7.5	10, 12.5	Incidence of tumors "quali- tatively and quantitatively similar" in test and controls	Not undertaken	1. Study poorly documented and methodologically unsound; female rats only tested. 2. Unacceptable as carcinogenicity test.
Davis, 1965	mouse/C3H	H and HE of unspec- ified purity	10	10	"Benign" hepa- tomas induced by H and HE	H and HE both hepatocarcino- genic	1. FDA data poorly documented. 2. FDA data statistically significant for tumor incidences. 3. Histological re-evaluation demon- strated hepato- carcinogenicity. 4. Hepatocarcino- genic effects statistically significant.
IRDC, 1973b	mouse/CD-1	Mixture of 25% H and 75% HE	1.0, 5.0, 10.0		Dose related nodu- lar hyperplasia at 5.0 and 10.0 ppm	Hepatocarcino- genic	1. IRDC data demon- strated statis- tically significant excess of nodular hyperplasias. 2. Histological re-evaluation found hepatocarcinomas. 3. Hepatocarcino- genicity statis- tically significant.

*Source: Adapted from tables compiled by the U.S. EPA (1980a) from data discussed by Epstein (1976).

5.0 and 7.0 mg/kg diet. Tumors included lymphomas, osteogenic sarcomas, carcinomas of the thyroid and subcutaneous fibrosarcomas. Liver lesions, described as the "chlorinated hydrocarbon" type, were observed with high incidence at the 7.0 and 10.0 mg/kg diet levels, but none were found at lower dose levels. These lesions were not believed to be neoplastic. The initial statistical analysis indicated that the incidence of tumors in treated rats was not significantly different from control incidence. Statistical re-analysis indicated significant differences in the incidences of tumors in treated female groups for any tumor ($p=0.034$), for malignant tumors in the 7.0 mg/kg diet group ($p=0.034$) and for any tumor in the 10.0 mg/kg diet group ($p=0.017$) (Epstein, 1976). The types of statistical tests used in these analyses are not detailed.

In another study by the Kettering Laboratory (Witherup et al., 1959), heptachlor epoxide was administered in the diet to groups of 25 male and 25 female CFN rats at concentrations of 0.5-10.0 ppm (mg/kg diet) for 108 weeks. The rats were 7 weeks of age at the beginning of the test. Similar groups were maintained on heptachlor epoxide-free diets. Necropsies were performed on all rats sacrificed when moribund or at the end of the experiment. Data on mortality and signs of toxicity were discussed previously in the Effects of Subchronic and Chronic Exposures Section. Liver tumors, both malignant and benign, occurred with random frequency among treated groups; none were observed in controls. Benign tumors of the pituitary were seen in all male groups and in high frequency in all female groups. Malignant tumors of the brain and reproductive organs occurred randomly among treated groups. It was concluded that the incidence of all tumors was unrelated to heptachlor epoxide treatment. An independent statistical analysis indicated

that there were significant differences between treated and control groups for total tumors (Epstein, 1976). The histological slides from this study were re-examined by two pathologists, Reuber and Williams (Epstein, 1976). The incidences of hepatic carcinomas as diagnosed by these pathologists are presented in Table V-16. As seen in Table V-16, the incidences of hepatic carcinomas was significantly increased above control incidence at 5 and 10 mg/kg diet levels in female rats from Reuber's analysis and at the 10 mg/kg diet level in males by Williams's analysis, when nodular hyperplasia (considered to be a carcinogenic response by Williams) is combined with carcinoma.

Jolley et al. (1966) studied the carcinogenic potential of a 75:25 mixture of heptachlor:heptachlor epoxide in CD rats. Groups of 25 female rats were administered the mixture in dietary concentrations of 5.0-12.5 ppm (mg/kg diet) for 2 years. A group of 54 female rats served as controls. Data on mortality and signs of toxicity were discussed previously in the Effects of Subchronic and Chronic Exposures Section. Upon necropsy, spontaneous tumors, such as mammary adenomas or fibroadenomas, were observed with random frequency among treated and control groups. No malignant lesions of liver were observed.

In a study on the carcinogenicity of heptachlor and heptachlor epoxide by the Food and Drug Administration (Davis, 1965), groups of 100 male and 100 female C3H mice were maintained on diets containing 10 ppm (10 mg/kg diet) of the respective compound for 2 years. Similar groups served as controls. Data on mortality and signs of toxicity were discussed in the Effects of Subchronic and Chronic Exposures Section. Many mice were lost or

TABLE V-16

Incidence of Hepatic Carcinoma in CFN Rats Following Chronic Dietary Exposure to Heptachlor Epoxide as Diagnosed by Reuber and Williams^a

Group (mg/kg diet)	Reuber		Williams	
	Incidence ^b	%	Incidence ^{b,c}	%
MALES				
0	1/24	4	0/24	0
0.5	1/22	5	1/23	4
2.5	0/19	0	0/21	0
5.0	1/23	4	1/22	5
7.5	1/25	4	1/25	4
10.0	4/22	8	5/22	3 ^f
FEMALES				
0	0/17	0	4/17	4
0.5	3/22	4	5/20	5
2.5	3/18	7	2/18	1
5.0	7/22	2 ^d	3/22	4
7.5	3/21	4	5/23	2
10.0	5/19	6 ^e	2/19	1

^aSource: Epstein (1976) review of Witherup et al., 1959

^bIncidence is expressed as $\frac{\text{number of tumor-bearing rats}}{\text{number of rats examined}}$

^cThe incidence of carcinoma is combined with the incidence of nodular hyperplasia since Williams stated: "...hyperplastic nodules are induced only by carcinogens...evidence of carcinogenic effect on liver..." (Epstein, 1976).

^dSignificant at $p=0.00111$

^eSignificant at $p=0.0308$

^fSignificant at $p=0.019$

discarded, sacrificed for transplant purposes or died premature deaths, so that statistical evaluation of the data was not attempted. There was an -2-fold increase in benign liver lesions in treated animals over control values. Malignant liver tumors occurred with less frequency in treated than in control mice. Subsequent statistical analysis was performed, assuming that all missing control mice had tumors and all missing treated mice did not. These assumptions resulted in liver tumor incidences (benign and malignant) of 53/200 controls, 51/200 mice treated with heptachlor and 94/200 mice treated with heptachlor epoxide. The difference for heptachlor epoxide from control was significant ($p < 0.01$). Subsequently, Reuber re-evaluated the slides from the Davis (1965) study (see also Reuber, 1977b), with the following results for liver carcinoma incidences: 22/78 (28%) control males, 2/54 (4%) control females, 64/87 (73%) heptachlor males, 57/78 (73%) heptachlor females, 73/79 (92%) heptachlor epoxide males and 77/81 (95%) heptachlor epoxide females. Three other independent pathologists also reexamined slides from 20 mice, and each one concurred with Reuber's diagnoses in 19/20 (95%) instances. Statistical analysis of Reuber's findings resulted in highly significant increased incidence of liver carcinoma in all treated groups when compared with controls ($p = 5 \times 10^{-6}$ to $< 1 \times 10^{-9}$) (Epstein, 1976; Reuber, 1977b).

IRDC (1973b) administered a 75:25 mixture of heptachlor epoxide:heptachlor in the diet to groups of 100 male and 100 female CD-1 mice at concentrations of 1.0, 5.0 or 10.0 ppm (mg/kg diet) for 18 months. The mice were 7 weeks old at the beginning of the test. Similar groups were maintained on insecticide-free diets and served as controls. Data on survival and signs of toxicity were discussed in the Effects of Subchronic and Chronic Expo-

tures Section. Although the incidences of nodular hyperplasia were highly significant for 5.0 and 10.0 mg/kg diet level males and females when compared with controls, the incidences of hepatomas were lower among the higher dose groups than in the 1.0 mg/kg diet group and controls. Reuber re-examined the slides from the IRDC (1973b) study and found a greater incidence of hepatic carcinoma, with a corresponding decrease in the incidence of hyperplasia and hyperplastic nodules (Epstein, 1976). The incidences of liver carcinoma among the various groups are presented in Table V-17. Re-analysis of the IRDC (1973b) slides by five other pathologists resulted in a general consensus that the incidence of hepatic carcinoma was considerably under-diagnosed in the original analysis (Epstein, 1976).

Dogs were administered heptachlor expoxide in a diet containing various dose levels for 2 years (U.S. EPA, 1971 -- IRDC, unpublished report). A level of 1 ppm in diet caused no adverse effect on parameters measured. Dose-related changes in biochemical values related to liver function and microscopic changes in liver were noted at the higher dose levels (3, 5, 7 and 10 ppm in diet).

Cabral et al. (1972) published a paper in Italian, for which there is an abstract in English, on the carcinogenic effects of heptachlor administered to perinatal rats. This study was also reviewed by Epstein (1976), whose article was consulted for details not available in the abstract. A group of 95 Wistar rats, 10 days of age, was administered 96.8% pure heptachlor in corn oil by gavage in a total of five doses. Each dose of 10 mg heptachlor/kg bw was administered on alternate days. Controls consisted of 19 males and 49 females treated with corn oil alone. Many rats were lost because of

TABLE V-17

The Incidence of Hepatic Carcinoma in CD-1 Mice Following Chronic Dietary Exposure to Heptachlor Epoxide:Heptachlor (75%:25%)^a

Groups (mg/kg diet)	Incidence ^b	%
MALES		
0	0/62	0
1.0	2/68	3
5.0	18/68	26 ($p=2 \times 10^{-5}$)
10.0	52/80	65 ($p<1 \times 10^{-5}$)
FEMALES		
0	6/76	8
1.0	1/70	1
5.0	6/65	9
10.0	30/57	53 ($p=5 \times 10^{-5}$)

^aBased on Reuber's re-evaluation of IRDC (1973b). Slides as reported by Epstein (1976).

^bIncidence is expressed as $\frac{\text{No. of tumor-bearing mice}}{\text{No. of mice examined}}$

the high mortality of both treated and control rats and to an interim kill at 60 weeks. All surviving rats were sacrificed at 106-110 weeks. Tumor incidences, as reported by Epstein (1976), were based on the number of rats at risk for 20 weeks. Endocrine tumors were found in 14/19 (74%) control males, 22/27 (81%) control females, 27/30 (90%) treated males and 15/29 (52%) treated females. The incidences of non-endocrine tumors was 5/19 (26%) control males, 4/27 (15%) control females, 3/30 (10%) treated males and 9/29 (31%) treated females. An independent statistical analysis found that the incidence of endocrine tumors in male rats receiving a total of 50 mg/kg bw heptachlor was significantly increased over controls ($p=0.033$) (Epstein, 1976).

In the NCI (1977b) bioassay, the carcinogenicity of heptachlor was studied using Osborne-Mendel rats and B6C3F₁ mice. Technical grade heptachlor (Ca 73% heptachlor, 22% trans-chlordane and 5% monochlor) was administered in the diet to groups of 50 rats or mice of each sex for 80 weeks, followed by an observation period of 30 weeks for rats and 10 weeks for mice. TWA high and low-dose concentrations were 77.9 and 38.9 ppm (mg/kg diet), respectively, for male rats and 51.3 and 25.7 mg/m diet, respectively, for female rats. For mice, the TWA high-dose and low-dose concentrations were 13.8 and 6.1 mg/kg diet for males and 18.0 and 9.0 mg/kg diet, respectively, for females. The designs of the dose schedules were presented previously in Tables V-10 and V-11. Comprehensive histological examination was performed on major organs and gross lesions of all animals that died spontaneously, were killed when moribund or were killed at the end of the study. Histological examination was precluded in some cases because of cannibalism or autolysis. For the rat study, 10 rats of each sex served as matched controls and 60 rats of each sex served as pooled controls. For the

mouse study, there were 20 male and 10 female matched control mice and 100 male and 80 female pooled control mice. Mortality data and symptoms of toxicity were discussed in the Effects of Subchronic and Chronic Exposures Section.

In rats, the incidence of neoplastic liver lesions was greater after heptachlor treatment than that observed after chlordane treatment (NCI, 1977a). No hepatocellular carcinomas were observed in any of the rats. One cholangiocarcinoma was diagnosed in the liver of one low-dose male. Neoplastic nodules, considered by the investigators to represent a cancerous condition (NCI, 1977b), were observed in all treated and control groups of rats. For males, the incidences were: 2/58 (3%) pooled controls, 1/10 (10%) matched controls, 3/44 (7%) low-dose males and 6/49 (12%) high-dose males. For females, neoplastic nodules were observed in 5/59 (8%) pooled controls, 1/10 (10%) matched controls, 9/48 (18%) low-dose females and 5/46 (11%) high-dose females. However, no statistically significant dose-related trend for the neo-plastic nodules was found for either sex by the Fischer exact test or by the life-table method when compared with pooled or matched controls. Follicular-cell carcinomas of the thyroid were found among treated groups, although the incidence was not statistically significant. A statistically significant dose-related trend was found in females, but not in males, when the carcinomas were combined with adenomas, or when the incidence of carcinomas was compared with pooled controls. C-cell carcinomas were found in treated males, and one was found in a control female. No significant dose-related trends for C-cell carcinoma was found even when adenomas were added to the analyses and compared with any control group. As stated in the report (NCI, 1977b): "In the judgment of the pathologist, the nature, incidence and severity of the proliferative thyroid lesions were not

sufficient to indicate clearly a carcinogenic effect of heptachlor in rats." Pituitary adenomas were seen frequently but no statistically significant differences were found. Other tumor types, not considered to be treatment-related, were distributed randomly among treated and control groups. The incidence of the neoplastic liver nodules, the tumors of the thyroid and the pituitary adenomas, albeit not considered to be treatment-related, are presented in Table V-18.

For B6C3F₁ mice receiving heptachlor, hepatocellular carcinoma was the most frequently observed neoplasm. The incidence was significantly different when compared with matched controls to high-dose males ($p=0.001$ or $p=0.0007$ by two simple proportion analyses; $p=0.002$ by life-table method). The incidence in low-dose males was lower than in the control group. A highly significant ($p<0.0001$) dose-related increase in the incidence of hepatocellular carcinomas was found for female mice because of the difference between high and low-dose groups. The difference in hepatocellular carcinoma incidence between high-dose females and matched controls was also significant ($p<0.005$). Other tumor types were observed with low frequency among all groups. The incidence of hepatocellular carcinoma in mice is presented in Table V-19.

Mutagenicity.

Chlordane -- Chlordane (unspecified purity, technical grade and commercial formulations) has been tested for mutagenicity and related effects in a number of systems (Table V-20). Negative results have been obtained in reverse mutation assays using nine strains of Salmonella typhimurium and two strains of Escherichia coli with and without metabolic activation systems (Probst et al., 1981; Gentile et al., 1982). Chlordane was negative when

TABLE V-10

Incidence of Hepatic Neoplastic Nodules and Thyroid Carcinomas and Adenomas Following Chronic Dietary Administration of Heptachlor to Osborne-Mendel Rats^{a,b}

	Male Rats ^c				Female Rats ^c			
	Pooled Control	Matched Control	Low Dose	High Dose	Pooled Control	Matched Control	Low Dose	High Dose
LIVER								
Neoplastic nodule	2/58 (3)	1/10 (10)	3/44 (7)	6/49 (12)	5/59 (8)	1/10 (10)	9/48 (19)	5/46 (11)
THYROID								
Follicular-cell carcinoma	1/51 (2)	0/8 (0)	4/38 (11)	0/38 (0)	1/58 (2)	1/9 (11)	2/49 (5)	5/38 (13)
Follicular-cell adenoma	3/51 (6)	1/8 (13)	7/38 (18)	3/38 (9)	2/58 (4)	0/9 (0)	1/43 (2)	10/38 (26)
C-cell carcinoma	1/51 (2)	0/8 (0)	3/38 (9)	1/38 (3)	5/58 (9)	1/9 (11)	0/43 (0)	0/38 (0)
C-cell adenoma	3/51 (6)	0/8 (0)	4/38 (11)	1/38 (3)	7/58 (12)	2/9 (22)	7/43 (16)	3/38 (9)
PITUITARY								
Adenoma	4/48 (29)	4/7 (57)	6/42 (19)	8/34 (24)	33/52 (63)	3/6 (50)	9/39 (23)	8/34 (21)

^aSource: NCI, 1977b

^bTumor incidence expressed as no. of tumor-bearing rats/no. of tissues examined.

^cThe numbers in parentheses are the incidences expressed as percents.

TABLE V-19

Incidence of Hepatocellular Carcinoma in B6C3F₁ Mice Following
Chronic Dietary Exposure to Heptachlor^a

Groups (mg/kg diet)	Incidence ^b	%
MALES		
0 (pooled)	17/92	18
0 (matched)	5/19	26
6.1	11/46	24
13.8	34/47	72
FEMALES		
0 (pooled)	3/78	4
0 (matched)	2/10	20
9.0	3/47	6
18.0	30/42	71

^aSource: NCI, 1977b

^bIncidence expressed as $\frac{\text{No. of tumor-bearing mice}}{\text{No. of tissues examined}}$

TABLE V-20

Mutagenicity Testing of Chlordane

Assay	Indicator/ Organism	Compound and/or Purity	Application	Concentration or Dose	Activating System	Response	Comment	Reference
Reverse mutation	<i>Salmonella</i> <i>typhimurium</i> G46, TA1535, TA1000, C3076, TA1537, B3052, TA1538, T:98	unspecified purity	plate incorporation	four gradient plates to give a 10-fold concen- tration range/ plate up to 10,000-fold range	±S9	-	- in all tester strains at all concen- trations	Probst et al., 1981
Reverse mutation	<i>S. typhimurium</i> TA1535, TA1537, TA1538, TA98, TA100	commercial formulation	plate incorporation	NR	±S9	-	- in all strains	Gentile et al., 1982
Reverse mutation	<i>S. typhimurium</i> TA1535, TA1537, TA1538, TA98, TA100	technical grade	plate incorporation	NR	±S9	-	- in all strains	Gentile et al., 1982
Reverse mutation	<i>Escherichia</i> coli WP2, WP2 urvr	unspecified purity	plate incorporation	four gradient plates to give 10-fold concen- tration range/plate up to 10,000-fold range	±S9	-	- in both strains at all concentrations	Probst et al., 1981
Mitotic gene conversion	<i>Saccharomyces</i> <i>cerevisiae</i> B4	commercial formulation	incorporated into log-phase cell suspension	NR	±S9	±	+ with meta- bolic activa- tion in a dose- related manner	Gentile et al., 1982
Mitotic gene conversion	<i>S. cerevisiae</i>	technical grade	incorporated into log-phase cell suspension	NR	±S9	±	+ with S9 activation in a dose- related manner	Gentile et al., 1982
Reverse mutation	<i>Zea mays</i> W22 homozygous for wx-C allele	commercial formulation	applied prior to seedling emergence	2.24 kg/ha	NA	+	none	Gentile et al., 1982

TABLE V-20 (cont.)

Assay	Indicator/ Organism	Compound and/or Purity	Application	Concentration or Dose	Activating System	Response	Comment	Reference
UDS	adult rat primary hepato- cytes	purity unspecified	cell culture incorporation	8 doses 0.5-1000.0 nmoles/ml	NA	-	chlordane was toxic to cells at >100 nmoles/ ml	Probst et al., 1981
UDS	adult male rat primary hepato- cytes	purity unspecified	applied to cover- slip cell cultures	10^{-6} - 10^{-1} M	NA	-	chlordane was toxic to cells at > 10^{-4} M	Maslansky and Williams, 1981
UDS	adult male mouse primary hepatocytes	purity unspecified	applied to cover- slip cell cultures	10^{-6} - 10^{-1} M	NA	-	chlordane was toxic to cells at > 10^{-4} M	Maslansky and Williams, 1981
UDS	adult male hamster primary hepatocytes	purity unspecified	applied to cover- slip cell cultures	10^{-6} - 10^{-1} M	NA	-	chlordane was toxic to cells at > 10^{-4} M	Maslansky and Williams, 1981
Dominant lethal	mouse CD-1	technical grade	gavage or i.p. injection	0, 50, 100 mg/kg bw	NA	-	- at all concentrations	Arnold et al., 1977
Dominant lethal	mouse CD-1	commercial formulation	gavage or i.p. injection	0, 50, 100 mg/kg bw	NA	-	- at all concentration	Arnold et al., 1977
UDS	SV-40 trans- formed human fibroblasts (VA-4)	unspecified purity	cell culture incorporation	1, 10, 100 and 1000 μ M	$\pm$ S9	- +	+ at each dose (<0.05) only without metabolic activation; >3-fold over control at 1 μ M	Ahmed et al., 1977
Inhibition of HELA cell growth	cells	Ortho Klor 74 (44.4% chlordane)	cell culture incorporation	0.0075-0.200 μ g/ml	NA	+	99-100% inh- bition with all concentra- tions tested	Blevins and Sholes, 1978

NR = Not reported; NA = Not applicable; UDS = Unscheduled DNA synthesis; i.p. = Intraperitoneal

assayed for unscheduled DNA synthesis in rat, mouse and hamster primary hepatocyte cultures. Concentrations of $>10^{-4}$ M chlordane were toxic to the cells (Probst et al., 1981; Maslansky and Williams, 1981). Commercial and technical grades of chlordane were negative in the dominant lethal assay in CD-1 mice following either intragastric or intraperitoneal administration (Arnold et al., 1977).

Positive results were obtained for mitotic gene conversion assays in Saccharomyces cerevisiae with but not without metabolic activation (Gentile et al., 1982). Ahmed et al. (1977) reported a >3 -fold increase ($p<0.05$) over controls in unscheduled DNA synthesis by SV-40 transformed human fibroblast cultures (VA-4) treated with 1 μ M chlordane, but only in the absence of the S-9 metabolic activating fraction. Ortho-Klor 74 (44.4% chlordane commercial formulation) inhibited by 99-100% the growth of HeLa cells at concentrations of 0.0075-0.200 μ l/l (Blevins and Sholes, 1978). Finally, chlordane was positive in a reverse mutation assay using a strain of Zea mays homozygous for wx-allele (Gentile et al., 1982). This assay in a plant was included for the sake of completeness since it was positive. Further details of these assays are given in Table V-20.

Heptachlor and Heptachlor Epoxide -- Heptachlor has been tested for mutagenicity in bacteria, yeast, maize, fruit flies, mice and rats, and for unscheduled DNA synthesis in mammalian cell cultures (Table V-21). Negative results have been obtained in the reverse mutation assay in 10 strains of Salmonella typhimurium and three strains of Escherichia coli for heptachlor tested with and without metabolic activation (Moriya et al., 1983; Probst et al., 1981; Marshall et al., 1976) and in the rec assay in two strains of Bacillus subtilis in which no activating system was used (Shirasu et al.,

TABLE V-21
Mutagenicity Testing of Heptachlor and Heptachlor Epoxide

Assay	Indicator/ Organism	Compound and/or Purity	Application	Concentration or Dose	Activating System	Response	Comment	Reference
Reverse mutation	<i>Salmonella typhimurium</i> TA100, TA98, TA1535, TA1537, TA1538	heptachlor/purity NR	plate incorporation	0-5000 µg/plate	±S9	-	- in all strains at all concen- trations	Moriya et al., 1983
Reverse mutation	<i>S. typhimurium</i> G46, TA1535, TA1000, C3076, TA1537, D3052, TA1538, TA98	heptachlor/NR	plate incorporation	four gradient plates to give 10-fold concentra- tion range/plate up to 10,000-fold range	±S9	-	- in all strains at all concentrations	Probst et al., 1981
Reverse mutation	<i>S. typhimurium</i> TA1535, TA1536, TA1537, TA1538	heptachlor/reference standard pure	plate incorporation	50-1000 µg/plate	±S9	-	- in all strains at all concentrations; toxic level >2500 µg/plate (-S9); >1000 µg/plate (+S9)	Marshall et al., 1976
Reverse mutation	<i>S. typhimurium</i> TA1535, TA1537, TA1538, TA98, TA100	heptachlor/technical grade	plate incorporation	range NR	±S9	±	maximum + in strains TA98 and TA100 with S9 at 10 µg/ plate	Gentile et al., 1982
Reverse mutation	<i>S. typhimurium</i> TA1535, TA1537, TA1538, TA98, TA100	heptachlor/commercial formulation	plate incorporation	NR	±S9	-	- in all strains	Gentile et al., 1982
Reverse mutation	<i>Escherichia coli</i> WP2 hcr	heptachlor/NR	plate incorporation	0-5000 µg/plate	±S9	-	None	Moriya et al., 1983

TABLE V-21 (cont.)

Assay	Indicator/ Organism	Compound and/or Purity	Application	Concentration or Dose	Activating System	Response	Comment	Reference
Reverse mutation	<u>E. coli</u> WP2, WP2 uvrA-	heptachlor/NR	plate incorporation	four gradient plates to give 10-fold concen- tration range/plate up to 10,000-fold range	$\pm$ S9	-	- in both strains at concentrations	Probst et al., 1981
Rec assay	<u>Bacillus</u> <u>subtilis</u> H17 Rec ⁺ , M45 Rec ⁻	heptachlor/NR	spot test	NR	none	-	none	Shirasu et al., 1976
Mitotic gene reversion	<u>Saccharomyces</u> <u>cerevisiae</u> D4	heptachlor/technical grade	incorporation into log-phase cell suspension	NR	$\pm$ S9	-	none	Gentile et al., 1982
Mitotic gene reversion	<u>S. cerevisiae</u> D4	heptachlor/commercial formulation	incorporation into log-phase cell suspension	NR	$\pm$ S9	-	none	Gentile et al., 1982
Reverse mutation	<u>Zea mays</u> W22 homozygous for wx-callelle	heptachlor/commercial formulation	applied prior to seedling emer- gence	1.12 kg/ha	NA	+	none	Gentile et al., 1982
Recessive lethal	<u>Drosophila</u> <u>melanogaster</u>	heptachlor/NR	injection into abdomen	5 x 10 ⁻⁴ % in water	NA	-	concentration was the highest sub- lethal concen- tration	Benes and Sram, 1969
UDS	adult rat primary hepato- cytes	heptachlor/NR	cell culture incorporation	0.5-1000 nmole/mg	NA	-	heptachlor was toxic to cells at >10 nmoles/mg	Probst et al., 1981

TABLE V-21 (cont.)

Assay	Indicator/ Organism	Compound and/or Purity	Application	Concentration or Dose	Activating System	Response	Comment	Reference
UDS	adult male rat primary hepatocytes	heptachlor/NR	applied to coverslip cell cultures	10^{-6} to 10^{-3} M	NA	-	heptachlor was toxic to cells at $>10^{-4}$ M	Maslansky and Williams. 1981
UDS	adult male mouse primary hepatocytes	heptachlor/NR	applied to coverslip cell cultures	10^{-6} to 10^{-3} M	NA	-	heptachlor was toxic to cells at $>10^{-4}$ M	Maslansky and Williams. 1981
UDS	adult male hamster primary hepatocytes	heptachlor/NR	applied to coverslip cell cultures	10^{-6} to 10^{-3} M	NA	-	heptachlor was toxic to cells at $>10^{-4}$ M	Maslansky and Williams. 1981
Dominant lethal	rat/3 genera- tions	heptachlor/NR	diet for 3 generations	1 and 5 ppm (mg/kg diet)	NA	+	increased number of resorbed fetuses	Cerey et al., 1973
Chromosome aberration	rat bone marrow	diet for 3 genera- tions	1 and 5 ppm (mg/kg diet)	NA	NA	+	increased numbers of abnormal mito- ses, chroma- tids, pulver- izations and translocations	Cerey et al., 1973
Dominant lethal	mouse CD-1	heptachlor/technical grade	gavage or i.p. injection	0, 7.5 and 15 mg/kg bw	NA	-	- at either dose by either route	Arnold et al., 1971
UDS	SV-40 trans- formed human fibroblasts	heptachlor/NR	cell cultures incorporation	100, 1000 μ M	$\pm$ SS	$\pm$	+ with S9 at both concen- trations ($p < 0.05$)	Ahmed et al., 1977

TABLE V-21 (cont.)

Assay	Indicator/ Organism	Compound and/or Purity	Application	Concentration or Dose	Activating System	Response	Comment	Reference
Reverse mutation	<i>S. typhimurium</i> TA100, TA98, TA1535, TA1537, TA1538	heptachlor epoxide/NR	plate incorporation	0-5000 µg/plate	±S9	-	- for all strains at all concentrations	Maruya et al., 1983
Reverse mutation	<i>S. typhimurium</i> TA1535, TA1536, TA1537, TA1538	heptachlor epoxide/ Reference Standard pure	plate incorporation	50-1000 µg/plate	±S9	-	toxic level >2500 µg/plate (-S9); >1000 µg/plate (+S9)	Marshall et al., 1976
Reverse mutation	<i>E. coli</i> MP2 hcr	heptachlor epoxide/NR	plate incorporation	0-5000 µg/plate	±S9	-	none	Maruya et al., 1983
Recessive lethal	<i>B. melanoqaster</i>	heptachlor epoxide	injection into abdomen	2.5 x 10 ⁻⁴ % in water	NA	-	concentration was the high- est sublethal concentration	Beres and Sram, 1969
Dominant lethal	mouse CD-1	heptachlor epoxide: heptachlor (75:25)	gavage or i.p. injection	0, 7.5 and 15 mg/kg bw	NA	-	- at either dose by either route	Arnold et al., 1977
UDS	SV-40 trans- formed fibre- blasts	heptachlor epoxide/NR	cell culture incorporation	10, 100, 1000 µM	±S9	±	± with S9 at each concen- tration (p<0.05)	Ahmed et al., 1977

NR - Not reported

NA - Not applicable

UDS - Unscheduled DNA synthesis

i.p. - intraperitoneal

1976). Gentile et al. (1982), however, reported a maximum positive response for reverse mutation in strains TA98 and TA100 of S. typhimurium at 10 µg technical grade heptachlor/plate with S-9 activation. Negative results were obtained in these strains when a commercial formulation of heptachlor was tested. Heptachlor, whether technical or commercial grade, was also negative for mitotic gene reversion in Saccharomyces cerevisiae in the presence and in the absence of S-9 (Gentile et al., 1982). Negative results were obtained in the recessive lethal assay in Drosophila melanogaster (Benes and Sram, 1969) and for unscheduled DNA synthesis by rat, mouse and hamster primary hepatocyte cultures (Probst et al., 1981; Maslansky and Williams, 1981). The dominant lethal assay in CD-1 mice receiving heptachlor either by gavage or intraperitoneal injection also gave negative results (Arnold et al., 1977); however, the dominant lethal assay in rats fed heptachlor at 1 and 5 mg/kg diet for three generations resulted in increased numbers of resorbed fetuses (Cerey et al., 1973). Increased incidences of chromosome aberrations were also noted in the bone marrow of these rats. Ahmed et al. (1977) reported positive results ($p < 0.05$) for unscheduled DNA synthesis in SV-40 transformed human fibroblasts (VA-4) with, but not without, the S-9 fraction at 100 and 1000 µM heptachlor.

Heptachlor epoxide was negative in reverse mutation assays in several strains of S. typhimurium and E. coli with and without metabolic activation (Moriya et al., 1983; Marshall et al., 1976) in the recessive lethal assay in D. melanogaster (Benes and Sram, 1969) and in the dominant lethal assay in CD-1 mice (Arnold et al., 1977). Ahmed et al. (1977) reported that heptachlor epoxide was positive in inducing unscheduled DNA synthesis in the SV-40 transformed human fibroblasts at concentrations of 10, 100 or 1000 µM in the presence, but not the absence, of S-9.

Teratogenicity and Other Reproductive Effects.

Chlordane -- Ingle (1952) found no fetotoxic or teratogenic effects in neonatal Osborne-Mendel rats following in utero exposure to technical chlordane. Female rats, two each from groups receiving chlordane at continuous dietary levels of 5, 10, 30, 150 or 300 ppm (mg/kg diet), were mated, one during the 24th week and the other during the 48th week of treatment. No effects were noted on fetal mortality, litter size or health of the pups. Three pups from each litter were allowed to suckle from foster dams that had not been exposed to chlordane; another three from each litter remained with their treated mothers. No differences in growth rates of pups resulted from suckling with exposed or non-exposed dams at 5, 10 or 30 mg/kg diet levels. No differences in growth rates were noted when these pups were compared with control pups that had not been exposed either in utero or by suckling. Hyperexcitability and mild tremors were present in pups born to and nursed by dams at the 150 mg/kg diet level that were mated at 48 weeks. One male pup died. No symptoms of toxicity developed in equivalent pups nursed by non-treated foster mothers. Of pups born to 300 mg/kg diet level dams, those transferred to non-treated foster dams did not develop toxic symptoms; those remaining with their treated natural mothers (24-week mating) developed tremors after nursing for 4 days. One male and two female sucklings died. One weanling male died and was underweight. Mortality was 100% within 5 days of nursing by dams treated at the 300 mg/kg diet level (48-week mating). Pups born to untreated dams but nursed by treated dams were similar to the pups born to and nursed by dams treated at each respective dietary level.

Welch et al. (1971) studied the effect of chlordane on mouse fertility. Female Swiss-Webster mice were treated intraperitoneally with 25 mg/kg bw

technical chlordane in corn oil once a week for 3 weeks. Controls received only corn oil. The mice were mated on the last day of injection and killed before the gestational period was complete. No effect was observed on litter size for pregnancy; however, the number of mice that became pregnant was appreciably reduced. Of 104 control mice, ~40 (38%) became pregnant. Of 105 treated mice, ~17 (16%) became pregnant. Microsomes prepared from ovariectomized mice treated intraperitoneally with 25 mg/kg bw chlordane once a week for 3 weeks increased the metabolism of estradiol by 3-5 times the control level. Chlordane pretreatment also inhibited the in vivo uterotrophic action of estrogens 88-98%. Welch et al. (1971) also studied the effects of chlordane pretreatment on estrogenic effects in Sprague-Dawley rats. Female rats (19-20 days of age) were treated intraperitoneally with technical chlordane or transchlordane (>99%) in corn oil daily for 7 days. At 25 mg/kg bw/day of either compound, the in vitro metabolism of estrone- $4-^{14}C$ by hepatic microsomes prepared from the treated rats was significantly increased. Pretreatment with 10 or 50 mg/kg bw/day for 7 days with technical grade chlordane significantly enhanced the in vivo metabolism of intraperitoneally administered estrogens. The uterotrophic response of estrone was inhibited by 93 and 98% by technical and transchlordane, respectively. The inhibition was significant ($p < 0.05$) at ≥ 5 mg/kg bw/day of technical grade chlordane.

Heptachlor and Heptachlor Epoxide -- No data on the teratogenicity of heptachlor or heptachlor epoxide were available. Mestitzova (1967) studied the effect of heptachlor on the fertility of rats. Heptachlor (98.1% pure) was "administered with food" at an "applied dose" of 6 mg/kg bw to an unspecified strain of rats. It is unclear whether this was a daily dose or a total dose administered over the duration of the treatment. There was a

marked reduction in litter size in F_1 generations and in one F_2 generation. Suckling pups had a high rate of mortality. During the first week after birth a mean percent mortality of the exposed pups was 46% as compared to 12% in controls. Cataracts developed in treated adults as well as in pups.

Welch et al. (1971) studied the effect of heptachlor on the metabolism of estrogen by Sprague-Dawley rats. Immature female rats (19-20 days of age) were treated intraperitoneally with heptachlor (>99% pure) at 10 mg/kg bw/day for 7 days. The in vitro metabolism of estrone-4- ^{14}C by microsomes prepared from the treated rats was significantly enhanced 2.5-fold over control levels. Pretreatment of rats by the same dose schedule inhibited the in vivo uterotrophic response of estrone by 67%.

Summary of Health Effects in Animals

Chlordane. Values for acute oral LD_{50} s of chlordane range from 83 mg/kg bw for cis-chlordane (Podowski et al., 1979) to 560 mg/kg bw for technical grade chlordane (Ambrose et al., 1953), when administered in lipophilic vehicle. Gaines (1960) determined an oral LD_{50} of 335 mg/kg bw for male and 430 mg/kg bw for female Sherman rats. Mice had about equal sensitivity (LD_{50} = 390 mg/kg bw), while hamsters were much less sensitive (LD_{50} 1720 mg/kg bw) (Gak et al., 1976). Neonatal rats were less sensitive than adults with an intraperitoneal LD_{50} of 1121 mg/kg bw (Harrison, 1975). Cis-chlordane was more toxic than trans-chlordane to mice (Ivie et al., 1972). Symptoms of acute intoxication include CNS stimulation, as evidenced by irritability, tremors and convulsions (Boyd and Taylor, 1969; Stohlman et al., 1950; Hyde and Falkenberg, 1976). At sublethal doses, hepatic microsomal enzyme induction (Den Tonkelaar and Van Esch, 1974) and

enhanced activities of hepatic and kidney cortical gluconeogenic enzymes (Kacew and Singhal, 1973) were observed. Delayed onset of eye and vaginal opening occurred in neonatal mice treated subcutaneously with 0.075 mg/mouse (Talamantes and Jang, 1977).

Maximum tolerated subchronic dietary concentrations were 800 and 400 mg/kg diet for male and female Osborne-Mendel rats, respectively, and 40 and 80 mg/kg diet for male and female B6C3F₁ mice, respectively (NCI, 1977b). Subchronic dietary exposure to chlordane has resulted in changes in prostate homeostasis in rats (Shain et al., 1977). The no-effect level for microsomal enzyme induction was <5 mg/kg diet (Den Tonkelaar and Van Esch, 1974). Daily oral intake of 1.2 mg/kg bw chlordane by rats resulted in no histopathologic damage to lungs, heart, stomach, liver, kidneys, spleen or testes (DeLong and Ludwig, 1954). Ingle (1952) defined a no-effect level in Osborne-Mendel rats at 5 mg/kg diet chlordane for histopathological changes in liver, kidneys, lungs, pancreas, stomach, adrenals, thyroid, thymus, lymph, testes, ovaries, heart and spleen, as well as for food consumption, growth rate and mortality. A dietary level of 10 mg/kg diet of chlordane produced hepatocytomegaly and mild bile duct proliferation. At higher concentrations, progressively more severe histopathological damage to liver and other organs occurred. Increased incidences of hepatic hyperplasia and hepatocytomegaly in mice were reported in a carcinogenicity study (IRDC, 1973a), but histological re-evaluation of slides by independent pathologists indicated that the hyperplastic nodules were carcinomas (Epstein, 1976). The incidence of liver hyperplasia was not increased in mice or rats in the NCI (1977a) bioassay, but benign proliferative lesions in chlordane-treated mice were found by Becker and Sell (1979).

Chlordane has been studied for carcinogenicity in mice and rats. In an unpublished study (IRDC, 1973a) reviewed by Epstein (1976), the incidence of hepatic carcinomas in mice was not significantly increased when compared with controls. The histologic slides were re-evaluated by independent pathologists who concluded that what IRDC (1973a) diagnosed as hyperplastic nodules were hepatocellular carcinomas. The incidence was significantly increased over control at 25 and 50 mg/kg diet in mice (Epstein, 1976). An NCI (1977a) bioassay also found a highly significant dose-related increase in the incidence of hepatocellular carcinoma in male and female B6C3F₁ mice exposed to chronic dietary levels of chlordane ranging from 29.9-63.8 mg/kg diet. Chlordane was not a hepatic carcinogen in Osborne-Mendel rats (NCI, 1977a). Becker and Sell (1979) found that C57BL/6N mice, a strain that does not develop spontaneous liver lesions, developed primary hepatocellular carcinomas during chronic exposure to a chlordane:heptachlor (90:10) mixture at 25 and 50 mg/kg diet levels.

Negative results for mutagenicity of chlordane were obtained in nine strains of Salmonella typhimurium and two strains of Bacillus subtilis for reverse mutation with or without metabolic activation (Probst et al., 1981; Gentile et al., 1982); in rat, mouse and hamster primary hepatocyte cultures for unscheduled DNA synthesis (Probst et al., 1981; Maslansky and Williams, 1981); and in mice for the dominant lethal assay (Arnold et al., 1977). Positive results were obtained in Saccharomyces cerevisiae for mitotic gene conversion with, but not without, metabolic activation (Blevins and Sholes, 1978) and in maize for reverse mutation (Gentile et al., 1982).

Ingle (1952) found no fetotoxic or teratogenic effects in neonatal Osborne-Mendel rats born to dams that were continuously fed chlordane at

5-300 mg/kg diet. Pups born to and nursed by treated dams developed dose-related symptoms of toxicity. Pups nursed by non-treated dams did not develop toxic signs. Pups born to non-treated dams and nursed by treated dams were comparable with pups born to and nursed by treated dams. Welch et al. (1971) found that approximately half as many mated female mice, which had been treated intraperitoneally with three weekly doses of 25 mg/kg bw/week of chlordane, became pregnant, as did untreated mice. Chlordane pretreatment in mice and rats resulted in enhanced in vitro and in vivo metabolism of estrogen and inhibited the uterotrophic response to estrogen.

Heptachlor and Heptachlor Epoxide. Heptachlor is more toxic than chlordane to laboratory animals. Acute oral LD₅₀ values in rats for heptachlor range from 40 mg/kg bw for a commercial formulation (Ben Dyke et al., 1970) to 162 mg/kg bw for female Sherman rats for technical grade heptachlor (Gaines, 1960). Oral LD₅₀s were 70 mg/kg bw for mice and 105 mg/kg bw for hamsters (Gak et al., 1976). The intraperitoneal LD₅₀ for neonatal rats was 531 mg/kg bw, but was reduced to 133 mg/kg bw by pretreatment with phenobarbital. It was suggested that the enhanced metabolic capacity of the neonate after phenobarbital dosing resulted in the metabolism of heptachlor to the more toxic heptachlor epoxide (Harbison, 1975). The acute oral LD₅₀ for heptachlor epoxide in adult rats was ~60 mg/kg bw (NAS, 1977; Sperling and Ewinike, 1969; Podowski et al., 1979). Symptoms of acute intoxication include tremors, convulsions, paralysis and hypothermia (Hrdina et al., 1974; Yamaguchi et al., 1980).

Moderate liver damage, accompanied by increased levels of serum GPT and serum aldolase, was observed in rats following single (60 mg/kg bw) or repeated oral doses (7 or 12 mg/kg bw/day) for 3-14 days (Krampl, 1971);

however, tolerance appeared to develop at 28 days. Enhanced activities of gluconeogenic enzymes, such as pyruvate carboxylase and glucose-6-phosphatase, in rats treated with a 200 mg/kg bw dose (above the LD₅₀) was observed by Kacew and Singhal (1973). Dietary levels of 2-50 mg/kg diet for 14 days resulted in induction of hepatic microsomal enzymes (Den Tonkelaar and Van Esch, 1974).

Maximum tolerated subchronic dietary concentrations were 160 and 80 mg/kg diet for male and female Osborne-Mendel rats, respectively, and 40 mg/kg diet for male and female B6C3F₁ mice (NCI, 1977b). Subchronic dietary exposure to heptachlor has resulted in changes in prostate homeostasis in rats (Shain et al., 1977) and induction of hepatic microsomal enzymes (Kinoshita and Kempf, 1970).

A dose-related increase in liver weight in male and female mice treated with 1-10 mg/kg diet levels of a heptachlor:heptachlor epoxide (25:75) mixture was reported in an unpublished carcinogenicity study (IRDC, 1973b, reviewed by Epstein, 1976). Increased incidences of hepatocytomegaly and hyperplastic nodules were also reported to be significantly increased. The histologic slides from this study were re-evaluated by six independent pathologists. The overall interpretation was that many of the hyperplastic nodules diagnosed by IRDC (1973b) were actually carcinomas (Epstein, 1976). Epstein (1976) examined other unpublished reports of heptachlor and heptachlor epoxide carcinogenicity (Davis, 1965; Witherup et al., 1959) that reported increased incidences of hepatic hyperplasia or nodules. Re-evaluations by independent pathologists again resulted in an interpretation in favor of increased incidences of carcinomas rather than hyperplastic nodules. Other unpublished reports (Witherup et al., 1955; Jolley et al.,

1966) were reviewed by Epstein (1976) and describe increased incidences of hepatocytomegaly and hepatic hyperplasia in rats and mice after chronic feeding with heptachlor and heptachlor epoxide. The incidence of liver hyperplasia was not increased in mice or rats in the NCI (1977b) bioassay. Decreased body weight gain was observed in male and female rats treated at 77.9 and 51.3 mg/kg diet levels, respectively. Reuber (1977a), in re-evaluating the slides from the Davis (1965) study, found appreciable incidences of hepatic vein thrombosis and cirrhosis of the liver in male and female mice treated at 10 mg/kg diet levels of heptachlor or heptachlor epoxide for 2 years. These conditions were not observed in any of the 127 controls.

Epstein (1976) reviewed several unpublished reports of chronic dietary carcinogenicity studies of heptachlor and heptachlor epoxide in rats and mice (Davis, 1965; Witherup et al., 1955, 1959; Jolley et al., 1966; IRDC, 1973b) in an article based on a Statement of Suspension Testimony at EPA Hearing on Heptachlor/Chlordane. Epstein (1976) also presented results of independent statistical analyses and re-evaluations of histologic slides. Increased incidences of nodular hyperplasia were reported for mice after heptachlor or heptachlor epoxide exposure (Davis, 1965; IRDC, 1973b). Independent re-evaluation of slides resulted in statistically significant increased incidences of hepatocellular carcinoma in mice and rats (Epstein, 1976). The dietary levels of heptachlor or heptachlor epoxide in these studies were 0.5-12.5 mg/kg diet.

An NCI (1977b) bioassay also found statistically significant dose-related increased incidences of hepatocellular carcinoma in male and female

B6C3F₁ mice exposed to chlordane at dietary levels ranging from 9.0-18.0 mg/kg diet. Heptachlor was not a hepatic carcinogen in Osborne-Mendel rats in the NCI (1977b) study, although there was a greater incidence of neoplastic nodules in rats after heptachlor treatment than after chlordane treatment (NCI, 1977a). The incidences of follicular-cell and C-cell carcinomas in the thyroid of rats were not considered to be treatment-related (NCI, 1977b).

Heptachlor was negative for reverse mutation in 10 strains of Salmonella typhimurium and 3 strains of Escherichia coli with and without metabolic activation (Moriya et al., 1983; Probst et al., 1981); in 2 strains of Bacillus subtilis in the rec assay (Shirasu et al., 1976); in Saccharomyces cerevisiae for mitotic gene conversion in the presence of S-9 (Gentile et al., 1982); in Drosophila melanogaster for recessive lethality (Benes and Sram, 1969); for unscheduled DNA synthesis in rat, mouse and hamster primary hepatocyte cultures (Maslansky and Williams, 1981); and for the dominant lethal assay in mice (Arnold et al., 1977). Positive results were obtained in the dominant lethal assay in rats (Cerey et al., 1973) and for unscheduled DNA synthesis in SV-40 transformed human fibroblasts with metabolic activation (Ahmed et al., 1977).

Heptachlor epoxide was negative for reverse mutations in S. typhimurium and E. coli with and without metabolic activation (Moriya et al., 1983; Marshall et al., 1976); recessive lethal assay in D. melanogaster (Benes and Sram, 1969); and the dominant lethal assay in mice (Arnold et al., 1977). Heptachlor epoxide was positive for unscheduled DNA synthesis in SV-40 transformed human fibroblast cultures (Ahmed et al., 1977).

Heptachlor was reported to cause a marked reduction in litter size of rats when administered in the diet for several generations (Mestitzova, 1967). The dose in this study was not clearly defined.

VI. HEALTH EFFECTS IN HUMANS

Clinical Case Studies

Many clinical case studies describing the acute and chronic toxic effects of technical grade chlordane, containing heptachlor, are available in the literature. The three effects seen most frequently are central nervous system effects, blood dyscrasias and neuroblastoma. In an attempt to substantiate the toxicity associated with chlordane exposure in clinical case studies, Dwight DeLong subjected himself twice, during two separate time intervals, to 7% chlordane vapors for 15 minutes every 3 days for 12 weeks (DeLong and Ludwig, 1954). No treatment-related effects were detected during repeated medical examinations, described as thorough but not further specified by the authors.

CNS Effects Associated with Exposure to Chlordane and Heptachlor. Ten clinical case studies describing CNS effects, along with miscellaneous other effects, following oral, dermal and inhalation exposure to technical grade chlordane containing heptachlor are summarized in Table VI-1. These effects frequently included, but were not limited to, irritability, salivation, labored respiration, muscle tremors, convulsions and death with or without an immediately preceding period of deep depression.

Five of these case studies described toxic effects occurring after oral exposure. Several CNS effects were observed in a 32-year-old woman (Case No. 1) who had ingested 104 mg chlordane/kg bw (Derbes et al., 1955), and in an 18-year-old woman (Case No. 2) who had ingested 32 mg chlordane/kg bw (estimated to be 10 mg/kg after vomiting) (Dadey and Kammer, 1953). Similar effects on the CNS were observed following ingestion of estimated chlordane

TABLE VI-1

Summary of Cases with CNS Effects Associated with Exposure to Chlordane or Heptachlor,
Either Alone or in Combination with Other Agents

Case No.	Case Description	Route of Exposure	Dose	Formulation	Duration of Exposure	Possible Confounding Factors	Symptoms	Reference
1	32-year-old, chronically depressed, white, widowed woman, weighing 58 kg	oral (ingestion)	6 g chlordane (104 mg/kg bw)	toxiclor dust containing 5% chlordane and related chemicals and 95% talc	single exposure	none	Clinical symptoms included chemical burns of the mouth, severe hemorrhagic gastritis, shock, anuria, convulsions, death; at autopsy, pathological findings included cerebral congestion and edema, lower nephron nephrosis, confluent bronchopneumonia and uremia.	Derbes et al., 1955
2	18-year-old, white, female student weighing 50 kg	oral (ingestion)	1.6 or 32 mg/kg (estimated that 500 mg or 10 mg/kg bw was retained after vomiting)	40% oil solution containing 26% chlordane	single exposure	none	Starting at 1.5 hours after ingestion: nausea, vomiting, diplopia, blurred vision, twitching of the extremities, generalized convulsion, no abnormal blood or urinalysis values, costovertebral angle tenderness.	Dadey and Kammer, 1953
3	15-month-old, female child, weight 9 kg, and in good health before ingestion of chlordane	oral (ingestion)	Not quantified (estimated to be <100 mg or <11.1 mg/kg bw)	wettable powder (50% chlordane); 1 tablespoon chlordane diluted to 1 quart with water	single exposure	none	Generalized tremor, incoordination, ataxia, convulsions (alternating tonic and clonic phases); irregular respirations; increased pulse rate; dilated pupils; irritability; risus sardonius; opisthotonus, bilateral ankle clonus, and generalized hyperactive reflexes; mild hypochromatic anemia (not clearly associated with exposure in the literature).	Lensky and Evans, 1952

TABLE VI-1 (cont.)

Case No.	Case Description	Route of Exposure	Dose	Formulation	Duration of Exposure	Possible Confounding Factors	Symptoms	Reference
4	4-year-old, Spanish American, female child weighing 11 kg (24 pounds)	oral (ingestion)	Not quantified (based on GI absorption estimated to be ≥ 3.62 mg or ≥ 0.15 mg/kg bw)	45% chlordane emulsifiable concentrate	single exposure	none	Clonic convulsions; coordination loss; hypoflexia; increased excitability; sinus tachycardia and right axis deviation, as evidenced by ECG; increased body temperature (100.3°F) on the day after exposure; absence of spontaneous vomiting.	Aldrich and Holmes, 1969
5	20-month-old, white, male child, weighing 12.7 kg (28 pounds)	oral (ingestion)	Not quantified	oil solution containing 74% technical grade chlordane	single exposure	Subject had a hypochromic microcytic anemia, which is usually associated with a history of poor iron nutrition.	Vomiting; frightened appearance; short interrupted seizures (both general and focal) accompanied by brisk deep tendon reflexes in all extremities; transient increased body temperature; hemic heart murmurs; transient increased white blood cell count; abnormal serum alkaline phosphatase and thymol turbidity levels at 3 months after exposure.	Curley and Gerrettson, 1969
6	47-year-old, married, male nurseryman; considered to be a "fairly heavy pipe smoker" (tobacco)	dermal (skin contact with soil containing chlordane)	Not quantified	NR	2 years	concurrent exposure to DDT, hormone sprays and arsenic pesticides	Jacksonian and grand mal convulsions; EEG revealed a generalized dysrhythmia with no localizing features; liver function tests were normal.	Barnes, 1967

TABLE VI-1 (cont.)

Case No.	Case Description	Route of Exposure	Dose	Formulation	Duration of Exposure	Possible Confounding Factors	Symptoms	Reference
7	23-year-old woman, who had worked with chlorinated hydrocarbons (including chlordane) for 2 years preceding acute exposure	dermal	Not quantified	suspension of 25 pounds of DDT, 39 pounds of veisicol, AR 50 and 10 pounds of triton X-100	single exposure	occupational exposure to chlorinated hydrocarbons for 2 years before acute exposure, but with no symptoms of previous drug intoxication; acute exposure also included DDT, veisicol, AR 50 and triton X-100	Confusion; convulsions; at autopsy, nonspecific pathological changes were seen in the kidneys, brain and lungs.	Derbes et al., 1955
8 ^b	56-year-old male dentist in excellent health until ~5 months after initiation of exposure	dermal and inhalation	Not quantified	wettable powder; 74% technical grade chlordane diluted 1:90 with water	~3-5 hours over 2 months for dermal; ~300 hours over 5 months for inhalation; exposures began simultaneously	"occasional" use of 5 mg diazepam (valium) and 200 mg ethchlorvynol (placidyl) before and during exposure	Starting at ~5 months after initiation of exposure: tachycardia; night sweats; fatigue; shortness of breath; pale conjunctivae; presence of 53 gallop; occult blood in stool; persistent elevated body temperature (38.8-39.4°C) with prostration, chills, and sweat; pallor of skin and mucous membranes; cholestatic hepatitis.	Furie and Trubowitz, 1976
9	No case description in the literature	dermal	Not quantified	75.3% technical grade chlordane in petroleum distillate	NR	NR	dizziness, nausea and vomiting	Klemmer et al., 1977
10	3-month-old infant	dermal and/or inhalation	Not quantified	NR	NR	concurrent exposure to aldrin	lethargy	Klemmer et al., 1977

^aThis case is also discussed in Table VI-2 (Case No. 17).

^bThis subject handled pipe tobacco after handling soil contaminated with chlordane, probably resulting in inhalation exposure in addition to the known dermal exposure.

NR = Not reported

doses of <11.1 mg/kg by a 15-month-old infant (Case No. 3) (Lensky and Evans, 1952) and ≥ 0.15 mg/kg by a 4-year-old child (Case No. 4) (Aldrich and Holmes, 1969). Curley and Garretson (1969) observed CNS effects in a 20-month-old infant (Case No. 5), but the dose level was not quantified. Dose levels for exposures by the dermal and/or inhalation routes were not quantifiable (Barnes, 1967; Derbes et al., 1955; Furie and Trubowitz, 1976; Klemmer et al., 1977).

Blood Dyscrasias Associated with Exposure to Chlordane and Heptachlor.

Exposure to chlordane and heptachlor via skin contact and/or inhalation has been associated with several blood dyscrasias, including four cases of aplastic anemia (Infante et al., 1978; Klemmer et al., 1977), one case of refractory megaloblastic anemia (Furie and Trubowitz, 1976), one case of acute stem cell leukemia (Infante et al., 1978), one case of acute lymphoblastic leukemia (Infante et al., 1978) and one case of acute myelomonocytic leukemia (Infante et al., 1978). Exposures were a result of indoor or outdoor applications or a combination of the two. These clinical case studies are summarized in Table VI-2.

Three cases (Nos. 1, 2 and 5) were mixed exposure with other pesticides, which may also be associated with blood dyscrasias in humans.

In addition to the clinical case studies described in Table VI-2, Muirhead et al. (1959) reported one case of hemolytic anemia associated with exposure (route of exposure not specified) to chlordane, heptachlor, dieldrin and toxaphene, and five cases of aplastic anemia, of which two were associated with exposure (routes of exposure not specified) to chlordane and

TABLE VI-2

Summary of Cases of Blood Dyscrasias Associated with Exposure to Chlordane or Heptachlor, Either Alone or with Other Agents

Case No.	Case Description	Description of Exposure	Exposure to Other Agents	Type of Blood Dyscrasia	Reference
11	male, 15 years old	Dermal via contact with outdoor spray for 5 months and inhalation via indoor application of chlordane.	isotox (containing carbaryl)	aplastic anemia	Infante et al., 1978
12	male, 28 year old, self-employed realtor	Extensive use of a 75% chlordane formulation for 6 months, before onset of symptoms (exposure not otherwise specified).	diazinon, various paints, thinners	aplastic anemia	Infante et al., 1978
13	male, 68 years old	Use of chlordane outdoors and indoors (75% chlordane) more than once during the 3 years preceding the onset of symptoms.	undetermined	aplastic anemia	Infante et al., 1978
14	female, 9 years old	Exposure to chlordane applied to the interior of her home initially at the age of 4 months and annually thereafter.	none	acute stem cell leukemia	Infante et al., 1978
15	male, 23 years old, employed by a lawn care firm	Spraying lawns with chlordane and other pesticides for 3 years before onset of symptoms.	Barvel D, diazinon, Dursban, 2,4-D, paints, strippers, thinners	acute lymphoblastic leukemia	Infante et al., 1978
16	male, 37 years old	Use of a 40% chlordane formulation for 10 years before onset of symptoms, "frequent" use indoors of full strength (application with a paint brush) and several times/year outdoors of diluted chlordane.	none	acute myelomonocytic leukemia	Infante et al., 1978

TABLE VI-2 (cont.)

Case No.	Case Description	Description of Exposure	Exposure to Other Agents	Type of Blood Dyscrasia	Reference
17*	male, 56 years old, dentist	Exposure to 74% technical grade chloroform diluted 1:90 with water for ~3-5 hours skin contact over 2 months and ~300 hours by inhalation over 5 months; exposures began simultaneously.	"Occasional" use of 5 mg diazepam (valium) and 200 mg ethchlorvynol (placidyl) before and during exposure	refractory megaloblastic anemia	Furie and Trubowitz, 1976
18	engineer; no other details provided	Treated an area under a house with 250 gallons of 4.5% chloroform emulsion.	not specified	aplastic anemia	Klemmer et al., 1977

*This case is also discussed in Table VI-1 (Case No. 8).

heptachlor only and three to chlordane, heptachlor and unspecified other drugs. Also, Loge (1965) reported 12 cases of aplastic anemia with pancytopenia, of which three were associated with chlordane exposure only and nine in conjunction with other drugs, and one case of thrombocytopenia and two cases of leukopenia agranulocytosis associated with exposure to chlordane in conjunction with other drugs.

The American Medical Association, Council on Drugs, as reported in the Registry on Blood Dyscrasias, concluded that there is evidence that a "specific cause-effect relationship exists" between exposure to chlordane and resulting blood dyscrasias, particularly pancytopenia, thrombocytopenia, leukopenia and anemia (Anonymous, 1962).

Neuroblastoma Associated with Pre- and/or Postnatal Exposure to Chlordane and Heptachlor. Infante et al. (1978) described five cases of neuroblastoma in children with a pre- and/or postnatal history of exposure to technical grade chlordane containing ~38.5% chlordane and 3-7% heptachlor. These cases are summarized in Table VI-3. Nine additional cases of neuroblastoma were diagnosed during the same time period (December 1974 to February 1976) at the same pediatric hospital, but pre- and/or postnatal exposure to chlordane and heptachlor were not ascertained. That effects may be induced by prenatal exposure is consistent with the documented trans-placental transfer of chlordane in humans (Curley et al., 1969; Wasserman et al., 1972, 1974; Zavan et al., 1969).

TABLE VI-3

Cases of Neuroblastoma Associated with Exposure to Chlordane and Heptachlor*

Case No.	Case Description	Description of Exposure and Confounding Factors	Pathological Condition
19	female, age 2 years and 8 months	Technical grade chlordane was applied to both the interior and exterior of the 28-year-old mother's home during the first trimester of gestation. Application to the basement, where the mother spent 25-30 hours/week typing, included sealing chlordane in holes drilled in cement blocks. The house was not well ventilated, because of cold weather. The mother took two valium tablets and received general anesthesia (thiopental sodium, halothane and nitrous oxide) early in the pregnancy. The exterior of the house was sprayed with chlordane a second time one year later.	Neuroblastoma (Stage III)
20	male, age 4 years and 5 months	Technical grade chlordane was applied to both the interior and exterior of the home every 6 months from one year before conception. The mother took aspirin during pregnancy.	Neuroblastoma (Stage IV)

TABLE VI-3 (cont.)

Case No.	Case Description	Description of Exposure and Confounding Factors	Pathological Condition
21	female, age 4 years and 4 months	Technical grade chlordane was applied to the interior of the home when the girl was 23 months old.	Neuroblastoma (Stage IV)
22	male, age 3 years and 9 months	Technical grade chlordane was applied to both the interior and exterior of the home 2 years before the child was born and when he was 2 years old. Between the two applications of chlordane, application of a commercial pesticide was intermittent.	Neuroblastoma (Stage IV)
23	female, age 6 years and 5 months	Chlordane dust was applied to the exterior foundation of the house when the child was 3 years and 8 months old (autumn of the year) and again in the spring of the next year.	Neuroblastoma (Stage IV)

*Source: Infante et al., 1978

Epidemiological Studies

Wang and MacMahon (1979a) conducted a retrospective mortality study of 1403 white male workers employed for >3 months in the two United States plants that produce chlordane and heptachlor. The total number of deaths observed in the cohort was 113 as compared with 157 expected, yielding a standardized mortality ratio (SMR) of 72. The observed incidences of all types of cancer except lung cancer were less than those expected. Death from lung cancer occurred in 12 members of the cohort as compared with 9 expected deaths (SMR = 134); however, the observed incidence was not statistically significant. It is noteworthy that only one death from liver cancer was observed; chlordane has been reported to be hepatocarcinogenic to mice (see Carcinogenicity in Chapter V). While the increased incidence of cerebrovascular disease was statistically significant (17 observed versus 9.3 expected; SMR = 183), the total deaths observed from all diseases of the circulatory system were less than expected. Wang and MacMahon (1979a) concluded that the increased incidence of cerebrovascular disease was not correlated to length of exposure or latency and occurred only after termination of employment. Wang and MacMahon (1979b) did not find a statistically significant increased incidence of cerebrovascular disease in a prospective epidemiological study of 16,126 male pesticide applicators, some of whom had been exposed to chlordane and heptachlor. Wang and MacMahon (1979a) concluded that they found no evidence of carcinogenicity to man as a result of exposure to chlordane and heptachlor, but "the study population is too small and the period of follow-up too short to translate this into a statement that there is no excess risk of cancer associated with exposure in man."

Five other studies (Alvarez and Hyman, 1953; Ditraglia et al., 1981; Fishbein et al., 1964; Princi and Spurbeck, 1951; Klemmer et al., 1977) assessing human effects of occupational exposure to chlordane and heptachlor were done with small study populations and short follow-up periods. Alvarez and Hyman (1953) examined a cohort of 24 workers (age range = 21-49 years) employed in the manufacture of chlordane for 2 months to 5 years. No evidence of any abnormality in the liver, kidneys, skin, CNS or hemopoietic system were observed. Ditraglia et al. (1981) conducted a retrospective mortality study of 327 chlordane-exposed workers and 305 heptachlor-exposed workers employed for ≥ 6 months. The ranges of SMRs were 66-82 for all causes of death and 55-132 for respiratory cancer. There was no increase in cerebrovascular disease for either group, but an increased incidence of stomach cancer was observed for the chlordane-exposed group. Fishbein et al. (1964) did not observe evidence of toxicity in a group of 15 workers engaged in the manufacture and packaging of chlordane for 1-15 years; air concentrations were reported to be 0.0012-0.0017 $\mu\text{g}/\text{L}$. Princi and Spurbeck (1951) examined a cohort of 34 workers (age range = 21-60 years) employed in the manufacture of chlordane, aldrin and dieldrin for 11-37 months (mean of 28 months). Neither effects on the respiratory system, CNS, kidneys, liver or hemopoietic system nor allergic responses were observed. Klemmer et al. (1977) surveyed 285 individuals in Hawaii from 1967-1973 by annual questionnaire and physical examination for exposure to chlordane. Sixteen survey participants (1 orchid grower, 2 farmers and 13 pest control operators) were exposed to chlordane by inhalation or dermal routes (doses not quantified), of which five were also exposed to other pesticides. The symptoms for most exposures included headache, dizziness, fatigue, skin rash, nausea, vomiting, diarrhea, lacrimation and diaphoresis.

High Risk Subpopulations

There are no clear identifiable high risk subpopulations that are inherently susceptible to exposure to chlordane, heptachlor or heptachlor epoxide. Various blood dyscrasias have been associated with exposure to chlordane and heptachlor (Infante et al., 1978; Furie and Trubowitz, 1976; Klemmer et al., 1977). Because of the large number of agents associated with these blood dyscrasias and the small incidence of these conditions, Infante et al. (1978) hypothesized that an idiosyncratic mechanism for susceptible individuals may be involved, but that identification of members of this sensitive subpopulation would be virtually impossible.

Several groups may be more affected than the general population because of high exposure rather than intrinsic susceptibility to chlordane, heptachlor or heptachlor epoxide. These high exposure groups include exterminators; agricultural applicators; workers employed in the manufacture of chemicals; infants nursing on human milk because of maternal excretion of heptachlor epoxide and oxychlordane, the major mammalian metabolites of heptachlor and chlordane, respectively, in mothers' milk (Savage et al., 1973; Strassman and Kutz, 1977); the developing fetuses of women exposed to chlordane because of transplacental transfer (Curley et al., 1969; Wasserman et al., 1972, 1974; Zvon et al., 1969); consumers of fish and shellfish (especially freshwater); persons residing in houses after termite extermination treatment with chlordane and heptachlor.

Summary of Health Effects

DeLong and Ludwig (1954) did not observe treatment-related effects in a human exposed to 7% chlordane vapors for 15 minutes every 3 days for 12

weeks. The clinical case studies of acute exposure to chlordane and heptachlor document a pattern of CNS effects similar to that found in animals. The course of poisoning frequently includes irritability, salivation, labored respiration, muscle tremors, convulsions and death with or without an immediately preceding period of deep depression. These symptoms were seen at doses as low as 32 mg/kg bw (estimated to be 10 mg/kg bw after vomiting) in an 18-year-old female (Dadey and Kammer, 1953) and 104 mg/kg bw in a 32-year-old female (Derbes et al., 1955). Several blood dyscrasias, including four cases of aplastic anemia (Infante et al., 1978; Klemmer et al., 1977), one case of refractory megaloblastic anemia (Furie and Trubowitz, 1976), one case of acute stem cell leukemia (Infante et al., 1978), one case of acute lymphoblastic leukemia (Infante et al., 1978) and one case of acute myelomonocytic leukemia (Infante et al., 1978), have been associated with exposure to chlordane and heptachlor. Infante et al. (1978) also suggested an association with pre- and/or postnatal exposure to chlordane and heptachlor.

The epidemiological studies available examine relatively small population sizes and have short follow-up periods. Wang and MacMahon (1979a) found an increased incidence of lung cancer and a statistically significant increased incidence of cerebrovascular disease in a cohort of 1403 white male workers employed for >3 months in the production of chlordane and heptachlor.

VII. MECHANISMS OF TOXICITY

Mechanism of Neurotoxicity

As discussed in the Effects of Acute Exposure and the Effects of Sub-chronic and Chronic Exposures Sections in Chapter V, one of the most commonly observed effects of chlordane or heptachlor exposure was stimulation of the CNS. Several investigators have studied the mechanism for neurotoxicity. Hyde and Falkenberg (1976) studied the effect of chlordane on brain potentials in male Sprague-Dawley rats that were surgically implanted with electrodes for EEG monitoring. Chlordane in a Tween-80-saline vehicle was administered intraperitoneally at 0.15, 1.75 or 25.0 mg/kg bw/day for 48 days. Control rats received only vehicle. Dose-related changes in neuro-electrical activity (frequency, amplitude and waveform changes) occurred during exposure, with increasing incidence as the duration of exposure progressed. No outward signs of toxicity were observed. The EEG changes included a shift toward fast beta rhythms (>25 Hz), elevated amplitudes, reductions of delta (0.5-3 Hz) and theta (4-7 Hz) frequencies. Sinusoidal waves were replaced by sharp, complex discharges. During the last 6 days of chlordane injection, the rats were starved and EEGs monitored. Abnormally high voltage potentials that suggested lethal patterns were observed. It was postulated that food deprivation resulted in mobilization of fat depots of chlordane and/or metabolites which then became neuroconcentrated. These EEG disturbances did not return to normal when chlordane treatment was discontinued, suggesting the persistence of chlordane as a neurotoxin.

St. Omer and Ecobichon (1971) studied the time course of changes in acetylcholine content of rat brain with respect to the time course of toxic symptoms induced by heptachlor. Female Wistar rats with carotid artery

cannulation were injected with a single dose of 13.0 mg/kg bw of reference grade heptachlor in a peanut oil-lecithin emulsion. Vehicle injected rats served as controls. Groups of six rats were sacrificed at 5, 15, 25 and 30 minutes after injection and brain extracts were prepared for acetylcholine content determination. Animals not sacrificed after treatment had mild tremors progressing to severe tremors in 2-26 minutes. Within 10-20 minutes after treatment, there were episodes of running, rolling and leg-paddling, followed by mild tonic-clonic seizures and inactivity at 18-26 minutes. Death of chlordane-treated rats occurred within 2 hours. The average acetylcholine content of rat brain extracts was ~6.5 $\mu\text{g/g}$ wet tissue at 5 minutes (vehicle control value = 3.3 $\mu\text{g/g}$ and remained constant) and ~10.5 $\mu\text{g/g}$ at 15 minutes. Acetylcholine levels declined to ~9.5 $\mu\text{g/g}$ at 25 minutes and ~9.0 $\mu\text{g/g}$ at 30 minutes after injection, but remained elevated above control levels.

In similarly conducted experiments, St. Omer (1971) studied the time course of changes in ammonia and glutamine content of rat brain. The time course of toxic symptoms was as previously described (St. Omer and Ecobichon, 1971). The ammonia content of the brain increased significantly to 2-fold above vehicle control levels at 25 minutes after treatment ($p < 0.05$). The glutamine content was unaffected. The increased ammonia level coincided temporally with mild seizures in heptachlor treated rats. It was noted that the level of ammonium ions in the brain increase during states of CNS excitation. Ammonia in the brain is removed via conversion to glutamine. Since, following heptachlor treatment, it appeared that this conversion did not take place, the accumulation of ammonia without subsequent removal induced the neurotoxicity. St. Omer (1971) concluded that

heptachlor acted by a mechanism that interfered with the production or utilization of ammonia.

The biochemical mechanism of neurotoxicity of heptachlor epoxide was studied by Yamaguchi et al. (1979) with respect to inhibition of synaptic ATPases. Synaptosomal, microsomal, synaptic vesicle and synaptic membrane fractions were prepared from homogenates of brain from Sprague-Dawley rats. The sensitivities of various ATPases in these fractions to heptachlor epoxide in vitro, in decreasing order, were as follows: Ca^{2+} , Mg^{2+} -ATPase (66.8-76.9% inhibition) > Mg^{2+} -ATPase (31.2-48.9% inhibition) > Ca^{2+} -ATPase (26.5-48% inhibition) > Na^+ , K^+ -ATPase (0-18.4% inhibition). The inhibition of Ca^{2+} , Mg^{2+} -ATPase was greatest in the synaptic vesicle fraction (which contained synaptic membrane components). The inhibition of Ca^{2+} , Mg^{2+} -ATPase by heptachlor epoxide was shown to reduce the binding capacity of Ca^{2+} . It was suggested that the resulting increased level of Ca^{2+} in the presynaptic region promoted the release of neurotransmitter (e.g., acetylcholine) contained in the synaptic vesicles, which would result in the appearance of toxic symptoms.

This phenomenon was further studied (Yamaguchi et al., 1980) using rat brain synaptosomes and glutamate as the model excitatory neurotransmitter. The release of transmitter is controlled by synaptic vesicle concentration of Ca^{2+} . In a series of experiments, it was demonstrated that heptachlor epoxide stimulated the uptake and inhibited the release of Ca^{2+} by synaptosomes, leading to accumulation of Ca^{2+} . Heptachlor epoxide also reduced the uptake of Ca^{2+} by the endoplasmic reticulum. Thus, Ca^{2+} within the synaptosomes was more available to synaptic vesicles to trigger release of

transmitter. The sequestration of Ca^{2+} within the synaptosome was related to the observed inhibition of Ca^{2+} , Mg^{2+} -ATPase (Yamaguchi et al., 1979). To confirm the in vitro results, heptachlor epoxide was administered intraperitoneally to rats at 200 mg/kg bw in corn oil. Violent convulsions occurred within 5 hours, at which time the rats were killed and synaptosomes prepared. The Ca^{2+} , Mg^{2+} -ATPase within synaptosomes was inhibited and a 4-fold greater concentration of heptachlor epoxide was found in synaptosomes than in whole brain.

Folmar (1978) found that in vitro rat brain microsomal Na^+ , K^+ -ATPase was significantly inhibited by chlordane (82%) and heptachlor (72%), suggesting a role of this ATPase in the neurotoxicity of these insecticides.

Mechanisms of Effects on Endocrine and Reproductive Organs

In an attempt to explain the action of chlordane and heptachlor on ventral prostate homeostasis (e.g., increased androgen receptor site content, decreased protein content) observed in rats following subchronic pesticide feeding (see Effects of Subchronic and Chronic Exposures Section in Chapter V), Shain et al. (1977) studied 5α -dihydrotestosterone binding to androgen receptors in vitro. Chlordane and heptachlor were found to be poor inhibitors of 5α -dihydrotestosterone binding to the receptor of ventral prostate cytoplasmic extracts or tissue mince preparations when compared with the inhibition by parathion. It was concluded that 5α -dihydrotestosterone binding inhibition was not a good predictor of the pesticide effect on ventral prostate homeostasis. An alternative mechanism was not presented.

Welch et al. (1971) found that chlordane administered intraperitoneally to mice at a dose of 25 mg/kg bw once a week for 3 weeks resulted in decreased fertility (see Teratogenicity and Other Reproductive Effects Section in Chapter V). A possible mechanism was investigated. Microsomes prepared from ovariectomized mice that had been pretreated with chlordane increased the metabolism of estrogen to polar metabolites by 3-5 times the control level. In addition, the in vivo uterotrophic action of exogenous estrogen was 88-98% inhibited in mice pretreated with chlordane. Similar results were obtained in the in vivo and in vitro experiments conducted in rats with chlordane (cis- and trans-isomers) and heptachlor.

Enzyme Induction and Related Mechanisms

Karel and Saxena (1976) observed increased levels of blood glucose, serum protein, serum alkaline and serum acid phosphatases in gerbils that had been treated intramuscularly with chlordane (see the Effects of Acute Exposure and the Effects of Subchronic and Chronic Exposures Sections in Chapter V). These investigators speculated that the increased level of serum protein was a result of increased incorporation of amino acids into protein in the liver, with subsequent release into the blood stream. The increased level of serum alkaline phosphatase may have resulted in part from increased activity of osteoblasts and in part by release from necrotic liver cells. Release of acid phosphatase from degenerating liver cells would result in higher serum levels of this enzyme, as well. The increase in blood glucose was explained as follows. Chlordane treatment activated the sympathetic nervous system, resulting in adrenal medulla secretion of adrenaline, and this stimulated anterior pituitary secretion of ACTH. ACTH activity resulted in production of glucocorticoid hormones, which in turn enhanced gluconeogenesis.

Enhanced gluconeogenesis was also observed in liver and kidney cortex of rats following acute or subchronic exposure to chlordane, heptachlor and heptachlor epoxide (Kacew and Singhal, 1973; Kacew et al., 1973; Singhal and Kacew, 1976). The enhancement was evidenced by increased activities of pyruvate carboxylase, phosphoenolpyruvate carboxykinase, fructose-1,6-di-phosphatase and glucose-6-phosphatase, as well as increased levels of blood glucose, serum urea and decreased liver glycogen (see the Effects of Acute Exposure and the Effects of Subchronic and Chronic Exposures Sections in Chapter V for dose, routes, durations, etc.). These investigators found that treatment of rats with these pesticides also resulted in significantly elevated activities of adenylyl cyclase and levels of cyclic AMP in the liver and kidney cortex, suggesting that synthesis of cyclic AMP was enhanced (Kacew and Singhal, 1973). Treatment of rats intraperitoneally with cyclic AMP (2x100 mg/kg bw) resulted in significantly increased activities of the four gluconeogenic enzymes, increased blood glucose and serum urea levels, and decreased liver glycogen content. Cyclic AMP, therefore, mimicked the action of pesticides (Singhal and Kacew, 1976). A mechanism of enhanced gluconeogenesis was proposed whereby the insecticides react with an adenylyl cyclase receptor on the plasma membrane, thus stimulating the activity of the enzyme to synthesize cyclic AMP from ATP. Cyclic AMP, in turn, may bind with a protein kinase receptor, resulting in a cyclic AMP independent protein kinase that is translocated to the nucleus. The protein kinase then catalyzes the phosphorylation of nuclear proteins, which initiates transcription, resulting in de novo synthesis of messenger RNA that induces the synthesis of gluconeogenic enzymes.

Increased activities of microsomal enzymes (phosphorothioate detoxication, O-demethylase and N-demethylase) were observed in rats fed chlordane, heptachlor or heptachlor epoxide for 13 weeks (Kinoshita and Kempf, 1970).

Effects on Cellular Respiration

Settlemyre et al. (1974) reported that heptachlor stimulated the ATPase of mouse liver mitochondria, but did not stimulate mitochondrial oxidation of succinate (respiration) in the absence of ADP. Respiration was stimulated in the presence of ADP. Heptachlor had no effect on ATP synthesis in the presence of ADP. It was stated that these effects were contrary to those observed for uncouplers of oxidative phosphorylation, which are known to stimulate respiration in the absence of ADP or inorganic phosphate and prevent ATP synthesis. An explanation to account for the enhanced respiration with ADP present, that there was an "increased influx of ADP into the mitochondria," was rejected on the basis that uptake of ^{14}C -ADP did not occur. It was suggested that, on the basis of a concentration-related increase in the light-scattering response of mitochondria by heptachlor, the insecticide dramatically altered the mitochondrial membrane, resulting in the observed increase in respiration in the presence of ADP. Two possible mechanisms were proposed: increased membrane permeability to succinate or conformational changes that increased the activity of the respiratory chain.

The effects of chlordane, heptachlor and heptachlor epoxide on beef heart mitochondrial electron transport was studied by Pardini et al. (1971). Chlordane and heptachlor inhibited the succinoxidase system to 21.2 and 5.8%, respectively, of control values. Heptachlor epoxide was not inhibitory. The activity of mitochondrial NADH-oxidase was inhibited 5.8 and

10.3%, respectively, of control values. When TMPD, which when reduced by NADH non-enzymatically donates electrons to the electron transport chain at the step between cytochrome b and cytochrome c, was added, electron transport proceeded, despite the oxidase inhibition. These results indicated that chlordane and heptachlor inhibition of electron transport occurred "on the substrate side of cytochrome c," since the inhibition of NADH oxidase was bypassed by the addition of TMPD.

Synergism/Antagonism

Harbison (1975) pretreated neonatal Sprague-Dawley rats with intraperitoneal injections of phenobarbital before intraperitoneal injection of chlordane or heptachlor. The LD₅₀ of heptachlor in neonates not pretreated with phenobarbital was 531 mg/kg bw. Phenobarbital pretreatment reduced the LD₅₀ to 133 mg/kg bw. The chlordane LD₅₀ of 1121 mg/kg bw was reduced to 539 mg/kg bw. Since phenobarbital is a known inducer of microsomal enzymes, the enhanced toxicity may have resulted from an increased capacity of the neonate to biotransform these insecticides to more toxic metabolites.

Sperling and Ewinike (1969) reported that pretreatment of adult male rats with an oral dose of 1.8 g turpentine/kg bw/day for 3 days reduced the oral LD₅₀ of heptachlor from 112 to 70 mg/kg bw. The toxicity of heptachlor epoxide was not affected.

Microsomes prepared from rats pretreated with chlordane intraperitoneally with 25 mg/kg bw/day for 3 days and challenged with an intraperitoneal injection of CCl₄ (0.5 ml of 25% solution) had elevated levels of NADPH-

cytochrome c reductase when compared with non-pretreated controls, although the increase was not statistically significant (Stenger et al., 1975). The cytochrome P-450 levels were significantly depressed by CCl_4 challenge from prechallenge values. Hepatocellular necrosis was extensive followed by CCl_4 challenge in chlordane pretreated rats. Rats treated only with CCl_4 had less extensive liver damage. Thus, chlordane pretreatment potentiated the toxicity of CCl_4 , presumably by inducing microsomal enzymes that metabolize CCl_4 . That chlordane induced the microsomal enzymes was demonstrated by reduced zoxazolamine paralysis time in vivo and increased NADPH-cytochrome c reductase and cytochrome P-450 levels in microsomes from non-challenged rats.

Boyd and Taylor (1969) maintained 133 male weanling Wistar rats on a low protein diet (3.5% casein) for 28 days. Another group of 122 rats was fed a normal protein diet (26% casein), and a third group of 141 weanlings was fed a commercial lab chow (25% protein from fish, milk, soybean and ground oats). After the feeding period, the rats were fasted overnight and treated with technical grade chlordane by gavage in a range of eight doses. The LD_{50} of chlordane in the low-protein diet group was 137 ± 30 mg/kg bw, in the 26% casein group was 267 ± 44 mg/kg bw and in the commercial diet group was 311 mg/kg bw. Thus, a protein diet protected the rats from chlordane toxicity.

The effects of protein and the quality of protein on heptachlor toxicity in weanling rats was studied in a series of experiments (Webb and Miranda, 1973; Miranda and Webb, 1973, 1974; Miranda et al., 1973; Weatherholtz et al., 1969). When 10% protein diets were fed to weanling rats, heptachlor

(administered intraperitoneally) was more toxic when the protein was high quality casein than when the low quality protein, gluten, was used. At 18% protein, this effect was more pronounced (Webb and Miranda, 1973). Rats receiving the gluten diet had reduced body weight, low microsomal protein content and lower activities of heptachlor epoxidase than rats pair-fed the casein diet. Rats maintained on low protein (5% casein) diets were 3 times less susceptible to heptachlor toxicity than were rats pair-fed 20 or 40% casein-containing diets (Weatherholtz et al., 1969). It was suggested that at low protein, metabolism of heptachlor to heptachlor epoxide was inhibited. This hypothesis was tested using an inhibitor (SKF525-A) or an inducer (phenobarbital) of microsomal enzymes (Miranda et al., 1973; Miranda and Webb, 1974). These agents were administered to rats that were maintained on casein or gluten diets before administration of heptachlor or heptachlor epoxide. The results indicated that SKF525-A, rather than protecting the rats from heptachlor, resulted in higher mortality in the gluten-fed rats. Phenobarbital likewise increased the toxicity of heptachlor, as expected, if metabolism to heptachlor epoxide was enhanced. In the casein-fed rats, SKF525-A had little effect on heptachlor toxicity; phenobarbital protected the rats. In similar experiments with heptachlor epoxide, SKF525-A slightly increased the toxicity in gluten-fed rats and greatly increased mortality in casein-fed rats. Phenobarbital completely protected both casein- and gluten-fed rats.

Mechanism of Carcinogenicity

Maslansky and Williams (1981) found that chlordane and heptachlor were negative for unscheduled DNA synthesis in rat, mouse and hamster primary hepatocyte cultures (see Mutagenicity Section in Chapter V). They concluded

that, since the insecticides were not genotoxic, the carcinogenicity involved an epigenetic mechanism, perhaps involving a promotional effect on liver cells already predisposed. They based this conclusion on observations that chlordane and heptachlor induce tumors only in the liver and only in mice that readily develop spontaneous liver lesions. Conversely, Becker and Sell (1979) demonstrated that chlordane induced primary hepatocellular carcinoma in a strain of mice (C57BL/6N) that historically and concurrently did not have spontaneous liver tumors.

Summary

Several studies on the mechanism of neurotoxicity of chlordane and heptachlor were reviewed. Hyde and Falkenberg (1976) described EEG changes in rats during subchronic chlordane exposure. Deprivation of food caused brain potentials indicating lethality, perhaps by causing mobilization of stored chlordane and/or metabolites. Changes in acetylcholine (St. Omer and Ecobichon, 1971) and ammonia and glutamate (St. Omer, 1971) levels in the rat brain were followed with the time course of symptoms of heptachlor toxicity in rats. Maximum acetylcholine levels occurred just before the onset of mild tonic-clonic seizures. Increased ammonia content coincided temporally with mild seizures, while glutamate levels were unaffected by heptachlor treatment.

Yamaguchi et al. (1979, 1980) found that heptachlor epoxide inhibited Ca^{2+} , Mg^{2+} -ATPase in rat brain synaptic vesicles and increased the level of Ca^{2+} in the presynaptic region; they suggested that this promoted the release of neurotransmitter. A 4-fold greater concentration of heptachlor epoxide was found in synaptosomes than in the whole brains of rats that

developed violent convulsions after a high dose of heptachlor epoxide. Folmar (1978) found that chlordane and heptachlor inhibited rat brain microsomal N^+ , K^+ -ATPase.

Shain et al. (1977) concluded that 5α -dihydrotestosterone binding inhibition experiments were inadequate to predict the effects of heptachlor and chlordane on rat ventral prostate homeostasis. Welch et al. (1971) found that chlordane exposure resulted in decreased fertility of mice. Chlordane and heptachlor were found to enhance the in vitro metabolism of estrogen and to suppress the uterotrophic action of exogenous estrogen in vivo.

Enhanced gluconeogenesis was explained by an action of chlordane on the sympathetic nervous system stimulating secretion of adrenalin and ACTH, which in turn enhanced the production of glucocorticoid hormone (Karel and Saxena, 1976). Alternately, Singhal and Kacew (1976) proposed a direct interaction of chlordane, heptachlor and heptachlor epoxide with an adenylyl cyclase receptor on the cell membrane, resulting in cyclic AMP synthesis. Cyclic AMP may react with an intracellular receptor, producing a protein kinase that is translocated to the nucleus where transcription is triggered. The newly synthesized messenger RNA would then induce gluconeogenic enzymes. Microsomal enzymes were also observed to be induced by the insecticides (Kinoshita and Kempf, 1970).

Settlemyre et al. (1974) and Pardini et al. (1971) described the effects of heptachlor, heptachlor epoxide and chlordane on cellular respiration.

Phenobarbital potentiated the toxicity of chlordane and heptachlor in neonatal rats (Harbison, 1975). Turpentine potentiated the toxicity of heptachlor, but not heptachlor epoxide in adult rats (Sperling and Ewinike, 1969). Chlordane potentiated the toxicity of CCl_4 in rats (Stenger et al., 1975). A high-protein diet protected rats from chlordane toxicity (Boyd and Taylor, 1969). A low-protein and a poor-quality-protein diet protected rats from heptachlor toxicity (Webb and Miranda, 1973; Miranda and Webb, 1974; Miranda et al., 1973; Weatherholtz et al., 1969). Experiments on the effects of an inhibitor and an inducer of microsomal enzymes on the toxicity of heptachlor in the protein diet groups were equivocal.

Maslansky and Williams (1981) suggested that chlordane and heptachlor exert their carcinogenic effects by an epigenetic mechanism.

VIII. QUANTIFICATION OF TOXICOLOGICAL EFFECTS

The quantification of toxicological effects of a chemical consists of an assessment of the noncarcinogenic and carcinogenic effects. In the quantification of noncarcinogenic effects, an acceptable daily intake (ADI) is calculated. An adjusted acceptable daily intake (AAD I) and health advisory (HA) values for the chemical are then calculated to define the appropriate drinking water concentrations to limit human exposure. For ingestion data, this approach is illustrated as follows:

$$ADI = \frac{(\text{NOAEL or LOAEL in mg/kg/day}) (\text{body weight in kg})}{\text{Uncertainty/Safety Factor}} = \text{mg/day}$$

$$AAD I = \frac{ADI}{\text{Drinking water volume in l/day}} = \text{mg/l}$$

where:

NOAEL = no-observed-adverse-effect level

LOAEL = lowest-observed-adverse-effect level

Body weight = 70 kg for adult or 10 kg for child

Drinking water volume = 2 l/day for adults or 1 l/day for children

Uncertainty/Safety Factor = 10, 100 or 1000

Utilizing these equations, the following drinking water concentrations are developed for noncarcinogenic effects:

1. A 1-day HA for 10 kg child
2. A 1-day HA for 70 kg adult
3. A 10-day HA for 10 kg child
4. A 10-day HA for 70 kg adult
5. A lifetime AADI for a 70 kg adult

The distinctions made between the HA calculations (items 1-4) are associated with the duration of anticipated exposure. Items 1 and 2 assume a single acute exposure to the chemical. Items 3 and 4 assume a limited

period of exposure (possibly 1-2 weeks). The HA values will not be used in establishing a drinking water standard for the chemical. Rather, they will be used as informal scientific guidance to municipalities and other organizations when emergency spills or contamination situations occur. The AADI value (item 5) is intended to provide the scientific basis for establishing a drinking water standard based upon noncarcinogenic effects.

A NOAEL or LOAEL is determined from animal toxicity data or human effects data. For animal data, this level is divided by an uncertainty factor because there is no universally acceptable quantitative method to extrapolate from animals to humans. The possibility must be considered that humans are more sensitive to the toxic effects of chemicals than are animals. For human data, an uncertainty factor is also used to account for the heterogeneity of the human population in which persons exhibit differing sensitivities to toxic chemicals. An Office of Drinking Water (ODW) modification of the guidelines set forth by the National Academy of Sciences (NAS, 1977, 1980) are typically used in establishing uncertainty factors as follows:

- An uncertainty factor of 10 is used when good acute or chronic human exposure data are available and supported by acute or chronic toxicity data in other species.
- An uncertainty factor of 100 is used when good acute or chronic toxicity data identifying NOEL/NOAEL are available for one or more species, but human data are not available.
- An uncertainty factor of 1000 is used when limited or incomplete acute or chronic toxicity data are available or when only the acute or chronic toxicity data identify a LOAEL (but not NOEL/NOAEL) for one or more species are available.

The uncertainty factor used for a specific risk assessment is judgmental. Factors that cannot be incorporated in the NAS/ODW guidelines (NRC, 1977) for selection of an uncertainty factor, but must be considered, include the following: 1) the quality of the toxicology data, 2) the significance of the adverse effect, and 3) the existence of counterbalancing beneficial effects.

If toxicological evidence requires the chemical to be classified as a potential carcinogen, mathematical models are used to calculate the estimated excess cancer risks associated with the ingestion of the chemical by drinking water. The bioassay data used in these estimates are from animal experiments. In order to predict the risk for humans, these data must be converted to an equivalent human dose. This conversion includes correction for noncontinuous animal feeding, nonlifetime studies and for the difference in size. The factor that compensates for the size difference is the cube root of the ratio of the animal and human body weights. It is assumed that the average human body weight is 70 kg and that the average human consumes 2 l of water per day. The multistage model is then fit to the equivalent human data to estimate the risk at low doses. The upper 95% confidence limit of this estimate is used. Excess cancer risks can also be estimated using other models such as the one-hit model, the Weibull model, the logit model and the probit model. There is no basis in the current understanding of the biological mechanisms involved in cancer to choose among these models. The estimates of low doses for these models can differ by several orders of magnitude.

The scientific data base used to calculate and support the setting of risk rate levels has an inherent uncertainty. This is because the tools of scientific measurement, by their very nature, involve both systematic and random error. In most cases, only studies using experimental animals have been performed. There is thus uncertainty when the data are extrapolated to humans. When developing risk rate levels, several other areas of uncertainty exist, such as 1) incomplete knowledge concerning the health effects of contaminants in drinking water, 2) the impact of test animal age, sex and species and the nature of target organ systems examined on the toxicity study results, and 3) the actual rate of exposure of internal targets in test animals or humans. Dose-response data are usually only available for high levels of exposure, not for the lower levels of exposure for which a standard is being set. When there is exposure to more than one contaminant, additional uncertainty results from a lack of information about possible synergistic or antagonistic effects.

Existing Guidelines, Recommendations and Standards

On March 6, 1978, the U.S. EPA (43 FR 12372) cancelled registrations of most pesticide products containing chlordane and heptachlor as defined in the notice of Intent to Cancel (39 FR 41298). The exceptions are the "use of heptachlor or chlordane through subsurface ground insertion for termite control and the dipping of roots or tops of nonfood plants."

Water. The U.S. EPA (40 FR 11990) proposed to set Interim Primary Drinking Water Standards for chlordane at 0.003 mg/l and for heptachlor and heptachlor epoxide at 0.0001 mg/l for maximum contaminant levels.

In the final U.S. EPA Regulations (40 FR 59566), however, these levels were deleted because the EPA was involved in the suspension and cancellation proceedings.

The Federal Water Pollution Control Administration (1968) set permissible surface water criteria for public water supplies at 0.003 mg/l for chlordane and 0.018 mg/l for heptachlor and heptachlor epoxide. The criteria for fish and other aquatic life based on LC_{50} of 0.002 mg/l for chlordane and 0.0002 mg/l for heptachlor would be very low; therefore, it is not recommended that these compounds be used near a marine environment. The Water Quality Criteria for farmstead use were 0.003 mg/l for chlordane and 0.018 mg/ml for heptachlor and heptachlor epoxide.

U.S. EPA (1980b) determined 4.6 ng/l for chlordane as the water concentration corresponding to an increased lifetime risk of cancer of 10^{-5} . This determination was based upon the incidence of hepatocellular carcinoma in male CD-1 mice following chronic dietary exposure as diagnosed by Reuber in his re-evaluation of slides from an IRDC (1973a) study that was reviewed by Epstein (1976) and upon a bioconcentration factor of 14,100 in fish. U.S. EPA (1980a) determined 2.8 ng/l for heptachlor as the water concentration corresponding to an increased lifetime risk of 10^{-5} . This determination was based upon the incidence of hepatocellular carcinoma in male B6C3F₁ mice in the NCI (1977b) bioassay.

Food. FAO/WHO (1978) recommended a maximum acceptable daily intake (ADI) value of 1 μ g/kg bw for chlordane. A value of 0.5 μ g/kg bw was recommended for heptachlor (FAO/WHO, 1972).

The registration for all uses of chlordane and heptachlor/heptachlor epoxide on food crops has been cancelled (43 FR 12372); however, when these insecticides were in use, the tolerances for residues in or on raw agricultural commodities for chlordane (40 CFR 180.122) and heptachlor and heptachlor epoxide (40 CFR 180.104) are given below:

Section 180.122 Chlordane; tolerances for residues.

A tolerance of 0.3 part per million is established for residues of the insecticide chlordane (1,2,4,5,6,7,8,8-octachloro-2,3,3a,4,7,7a-hexahydro-4,7-methanoindene, containing not more than 1 percent of the intermediate compound hexachlorocyclopentadiene) in or on each of the following raw agricultural commodities: Apples, apricots, beans, beets (with or without tops) or beet greens alone, blackberries, blueberries (huckleberries), boysenberries, broccoli, brussels sprouts, cabbage, carrots, cauliflower, celery, cherries, citrus fruits, collards, corn, cucumbers, dewberries, eggplants, grapes, kale, kohlrabi, lettuce, loganberries, melons, nectarines, okra, onions, papayas, peaches, peanuts, pears, peas, peppers, pineapples, plums (fresh prunes), potatoes, quinces, radishes (with or without tops) or radish tops, raspberries, rutabagas (with or without tops) or rutabaga tops, squash, strawberries, summer squash, sweet potatoes, tomatoes, turnips (with or without tops) or turnip greens, youngberries.

Section 180.104. Heptachlor and heptachlor epoxide; tolerances for residues.

0.1 part per million in or on cabbage, lettuce, rutabagas, snap beans.

Zero in or on alfalfa, apples, barley, beets (including sugar beets), blackeyed peas, brussels sprouts, carrots, cauliflower, cherries, clover, corn, cottonseed, cowpeas, grain sorghum (milo), grapes, grass (pasture and range), kohlrabi, lima beans, meat, milk, oats, onions, peaches, peanuts, peas, pineapple, potatoes, radishes, rye, sugarcane, sweet clover, sweet potatoes, tomatoes, turnips (including tops), wheat.

Air. The American Conference of Governmental Industrial Hygienists (ACGIH, 1983) has adopted TWA-TLVs of 0.5 mg/m³ for chlordane and heptachlor in workroom air. Likewise, the U.S. Occupational Safety and Health Administration standard TLV for exposure in the workroom is 0.5 mg/m³ for

chlordan and heptachlor (29 CFR 1910.1000). Corresponding values for heptachlor are 0.5 mg/m³ in the Federal Republic of Germany and 0.01 mg/m³ in the USSR (Winell, 1975).

The U.S. National Research Council (NRC, 1982) has recommended an interim guideline for airborne chlordan and heptachlor in military housing of 5 and 2 µg/m³, respectively.

Chlordan

Noncarcinogenic Effects. There are several reports of accidental or intentional acute oral exposure of humans to chlordan for which a dose or an estimation of a dose were obtained. In one case, a 32-year-old woman who had ingested 104 mg chlordan/kg body weight died 10 days after exposure (Derbes et al., 1955). Another case of human exposure involved an 18-year-old woman who had ingested 32 mg chlordan/kg body weight (Dadey and Kammer, 1953). It was estimated that she retained 10 mg/kg bw after vomiting. She recovered spontaneously. Lensky and Evans (1952) reported a case of a 15-month-old girl, weighing 9 kg, who drank a small amount of a chlordan suspension. The investigators believed that the amount was <100 mg of chlordan (dose <11.1 mg/kg bw). The child developed tremors, ataxia, convulsions, risus sardonicus, ophisthotonos and other CNS disturbances. Among the symptoms of a 4-year-old girl weighing 11 kg who absorbed an estimated dose of ≥0.15 mg/kg bw chlordan were clonic convulsions, loss of coordination, hyporeflexia, excitability and sinus tachycardia (Aldrich and Holmes, 1969). In a similar case, a 20-month-old boy weighing 12.7 kg swallowed 74% technical grade chlordan (Curley and Garrettson, 1969). His symptoms included vomiting, convulsions and seizures. The investigators did

not calculate a dose. Some of the signs of toxicity observed may have been associated with the petroleum distillate used in the formulation of liquid commercial products of chlordane.

Much of the information on single oral exposures of laboratory animals to chlordane is concerned with lethality. For rats, reported acute oral lethal dose range from 83-430 mg/kg bw depending on the vehicle and whether technical or pure chlordane is administered (Podowski et al., 1979; Ambrose et al., 1953; Gaines, 1960; Boyd and Taylor, 1969). Gak et al. (1976) reported oral LD_{50} s of 390 mg/kg bw for mice and 1720 mg/kg bw for golden hamsters. Male and female neonatal Sprague-Dawley rats were less sensitive to intraperitoneal administration of chlordane (i.p. LD_{50} = 1121 mg/kg bw) than were adult males (i.p. LD_{50} = 343 mg/kg bw) (Harbison, 1975). Signs of acute chlordane poisoning included CNS disturbances such as tremors, convulsions, irritability, hyporeflexia, dyspnea, and ataxia, among others (Lehman, 1951; Gaines, 1960; Boyd and Taylor, 1969; Stohlman et al., 1950; Arnold et al., 1977). Histological examination of fatally poisoned rats revealed nephritis, hepatitis and vascular congestion (Boyd and Taylor, 1969).

However, sublethal doses have also been tested. Ambrose et al. (1953a,b) observed slight histologic changes in the liver (intracytoplasmic bodies at all dose levels) at doses of 6.25-25 mg/kg bw/day by gavage. These doses are considered Adverse-Effect levels (AEL). The next higher dose, 50 mg/kg bw/day resulted in death of 2 of 5 animals. This dose represents a Frank-Effect level (FEL). At exposures of 10-50 mg/kg diet for 14 days in groups of six male Wistar rats, statistically significant

microsomal enzyme induction (aniline hydroxylase, aminopyrine demethylase and hexabarbital oxidase) was observed when compared with controls (Den Tonkelaar and Van Esch, 1974). This exposure corresponds to doses of 1.0-5.0 mg/kg bw/day assuming that a young rat consumes food at a level of 10% of its body weight per day. Based on the effects observed these doses might represent NOAELs. These investigators did not look for other effects. The NOEL for enzyme induction was 5 mg/kg diet (0.05 mg/kg bw/day) for 14 days.

Subchronic dietary exposure of mice and rats to technical grade chlordane for 42 days resulted in increased mortality at 160 and 800 mg/kg diet, respectively (NCI, 1977a). These doses represent FELs. Histopathologic examinations of the animals at these dietary levels or below were not performed. Subchronic feeding of male rats with chlordane for 90 days at a dose of 19.5 mg/kg bw/day resulted in decreased mean weekly body weight gains compared with controls and changes in ventral prostate homeostasis (increased androgen receptor site content, decreased prostate protein, RNA and DNA contents) (Shain et al., 1977). This latter dose can be considered an AEL.

Chronic dietary exposure of male and female rats to chlordane at 0-1280 mg/kg diet for 400 days resulted in a variety of adverse changes. Increased mortality was observed in the two higher dose levels. Dietary levels at or above 80 mg/kg diet in both sexes yielded histopathologic liver changes (vacuolization and enlarged nuclei) that were dose-related. Liver weight was increased in female rats of the 10 mg/kg diet group (Ambrose et al., 1953a,b).

In studies designed to assess the carcinogenicity of chlordane in mice (IRDC, 1973a; NCI, 1977a), such effects as increased mean liver weight, decreased mean body weight and increased mortality were observed among treated groups at dietary levels ranging from 25-63.8 mg/kg diet. In addition, the incidences of hepatocellular carcinoma were significantly increased above controls in these studies. At 5 mg/kg diet in the IRDC (1973a) study, the only observed effects were increased mean liver weights and hepatocytomegaly in females.

Chlordane was not carcinogenic to Osborne-Mendel rats in the NCI (1977a) bioassay; however, tremors, clinical signs of toxicity, reduced mean body weight and increased mortality were observed in various male and female groups treated at 120.8-407.0 mg/kg diet for 80 weeks when compared with controls. Ingle (1952) described the dose-related effects of dietary levels of 5-300 mg/kg diet of chlordane in male and female Osborne-Mendel rats treated for 2 years. At 300 mg/kg diet, severe histopathological damage to liver, kidney, heart, adrenals, lung and spleen; increased mortality; tremors; and hemorrhaging were observed. These effects were less pronounced at 150 mg/kg diet. At 30 mg/kg diet, slight liver damage was observed. At 10 mg/kg diet, minor liver damage consisting of an occasional hypertrophic cell and minor bile duct proliferation was the only effect. No effects on food consumption, growth rate, mortality, no signs of toxicity and no histopathologic changes were observed at 5 mg/kg diet. All of the above doses, with the exception of the last one, represent AELs. The last dose represents a NOAEL.

An additional report by Vettorazzi (1975) describes a review by a WHO/FAO scientific panel of an unpublished study in dogs performed by Wazeter, 1967. In this study, the dogs were exposed to diets containing chlordane at levels of 0-30 mg/kg diet for 2 years. The review panel determined that an exposure of 3 mg/kg diet was a NOEL and reported that exposure to 15 or 30 mg/kg diet resulted in increased liver weight and histologic changes in the liver. These latter doses are considered AELs. Assuming that a dog consumes 2.5% of the body weight in food per day the dietary level that is a NOEL is equivalent to a dose of 0.075 mg/kg bw/day.

Ingle (1952) found no teratogenic or fetotoxic effects in neonatal rats following in utero exposure to technical grade chlordane from dams that had been mated in the study discussed above. He did observe toxic symptoms in pups nursed by dams exposed to 150 and 300 mg/kg dietary levels. Toxic symptoms also developed in pups not exposed in utero, but nursed by dams treated at these levels. Fertility was reduced to ~50% of control mice in female Swiss Webster mice treated intraperitoneally with three weekly doses of 25 mg/kg bw technical chlordane (Welch et al., 1971).

Quantification of Noncarcinogenic Effects.

1-Day and 10-Day HA -- Satisfactory dose-response data are not available from which a HA can be derived for 1 and 10 days. However, in order to have some guidelines available, a 10-day HA for chlordane may be derived based upon the Ambrose et al. study (1953a,b) in rats. The toxic effects resulting from daily gastric intubation of doses 6.25, 12.5, 25.0, 50.0, 100.0 or 200 mg/kg chlordane in rats for 15 days were histological changes in the liver of the treated animals (at all dose levels) and central nervous

system effects at higher dose levels. The minimal histopathological changes such as presence of abnormal intracytoplasmic bodies of various diameters were evident at dose levels of 6.25 mg/kg. Therefore, this dose level may be used in the development of a 10-day HA and a safety factor of 1000 may be applied in the calculation since data are for the animals rather than human. Accepting 6.25 mg/kg as the minimal effect dose, the HA is derived as follows:

1-Day HA = Insufficient data

Derivation of 10-Day HA --

For a child:

$$\begin{aligned} 10\text{-Day HA} &= \frac{(6.25 \text{ mg/kg/day}) (10 \text{ kg})}{(1000) (1 \text{ L/day})} = 0.0625 \text{ mg/L} \\ &= 0.063 \text{ mg/L} \end{aligned}$$

For an adult:

$$\begin{aligned} 10\text{-Day HA} &= \frac{(6.25 \text{ mg/kg/day}) (70 \text{ kg})}{(1000) (2 \text{ L/day})} = 0.219 \text{ mg/L} \\ &= 0.22 \text{ mg/L} \end{aligned}$$

where:

6.25 mg/kg/day = minimal effect level

10 kg = body weight for a child

70 kg = body weight for an adult

1000 = uncertainty factor -- intra- and interspecies variation and LOAEL

1 l/day = water consumption per day by a child

2 l/day = water consumption per day by an adult

It should be noted that this HA for short-term exposure is also applicable for a 1-day HA in view of the metabolism studies of Barnett and Dorough (1974) and Tashiro and Matsumura (1977).

Lifetime AADI -- Wazeter (1967), which was reported by Vettarazzi (1975), described a study in which a NOEL of 3 mg/kg of diet was obtained after a 2-year feeding study of chlordane in dogs. The chemical was of technical grade in this study. Although this study is unpublished, it has been reviewed by a panel of scientists for WHO/FAO. The NOAEL of 3 mg/kg diet is similar to the NOAEL observed by Ingle (1952) in rats of 5 mg/kg of diet containing γ -chlordane. An ADI can be derived from the study described by Vettarazzi (1975) in dogs where 3 mg/kg (0.075 mg/kg bw/day) is a NOAEL. The calculations for an ADI are:

$$ADI = \frac{(0.075 \text{ mg/kg/day}) (70 \text{ kg})}{(100)} = 0.0525 \text{ mg/day}$$

where:

0.075 mg/kg/day = No effect level

70 kg = Assumed weight of an adult

100 = Safety factor where no adverse health effect was observed

Using 0.0525 mg/day as the ADI and assuming the 70 kg adult consuming 2 l of drinking water per day, the AADI is calculated as follows:

$$\text{AADI} = \frac{0.0525 \text{ mg/day}}{2 \text{ l}} = 0.0263 \text{ mg/l} \approx 0.03 \text{ mg/l}$$

Assuming 20% relative source contribution from drinking water, the AADI would be:

$$\frac{(0.0525 \text{ mg/day}) (0.20)}{2 \text{ l}} = 0.005 \text{ mg/l}$$

A summary of the data used to calculate the recommended 1-day and 10-day HAs and the lifetime AADI for chlordane is provided in Table VIII-1.

Carcinogenic Effects. Several cases of neuroblastoma in children that had been exposed to technical grade chlordane pre- or postnatally, and several cases of leukemia associated with dermal or inhalation exposure to chlordane, have been reported (Infante et al., 1978). Quantification of exposure could not be made. A retrospective epidemiological study failed to find an increased incidence of most cancer types over expected numbers of 1403 white male workers employed in the manufacture of chlordane (Wang and MacMahon, 1979a). An exception was lung cancer; 12 members of the cohort died from lung cancer compared with 9 expected. Only one death from liver cancer occurred. Wang and MacMahon (1979b) also failed to find evidence of carcinogenicity in a prospective epidemiological study of 16,126 male pesticide applicators, although the follow-up period may have been too short to

TABLE VIII-1
Summary of Data Used to Derive HAs and AADIs for Chlordane

Criteria	Animal Dose	Duration	Effect	Value of HA or AADI		Reference
				Child	Adult	
1-day HA	--	Insufficient	Data	--	--	--
10-day HA	rat, 6.25 mg/kg/day	15 days	Intracytoplasmic bodies of various diameters within hepatocytes	0.063 mg/L	0.22 mg/L	Ambrose et al., 1953a,b
AADI	dog	2 years	NOAEL, higher doses caused histopathological changes	--	0.030 mg/L ^a 0.005 mg/L ^b	Vettarazzi, 1975
Increased lifetime risk level for a 70 kg adult		--	Hepatic carcinomas	10 ⁻⁴ 2.18 µg/L	10 ⁻⁵ 0.218 µg/L 0.0218 µg/L	U.S. EPA, 1980b

^aAssuming 100% contribution being from water

^bAssuming 20% contribution being from water

indicate that no excess risk existed. Ditraglia et al. (1981) found an increased incidence of stomach cancer in a retrospective mortality study among 327 chlordane-exposed workers.

Chlordane has been studied for carcinogenicity in mice and rats. In an unpublished study (IRDC, 1973a) reviewed by Epstein (1976) the incidence of hepatic carcinoma in male and female CD-1 mice receiving dietary concentrations of 0, 5, 25 and 50 mg/kg diet was not significant. The histologic slides were re-evaluated by Reuber, whose diagnoses were supported by three other independent pathologists. The re-analysis resulted in the following incidences of hepatocellular carcinoma: 3/33 (9%), 5/55 (9%), 41/52 (79%) and 32/39 (82%) for 0, 5, 25 and 50 mg/kg diet males, respectively, and 0/45 (0%), 0/61 (0%), 32/50 (64%) and 26/37 (70%) for females of each respective group. The incidences at 25 and 50 mg/kg diet were statistically significantly different from control for both males and females ($p < 1 \times 10^{-5}$).

In an NCI (1977a) bioassay the incidence of hepatocellular carcinoma in male and female B6C3F₁ mice followed a statistically significant dose-related trend ($p < 0.001$). Male mice received TWA dietary concentrations of chlordane of 0, 29.9 and 56.2 mg/kg diet for 80 weeks and had incidences of hepatocellular carcinoma of 2/18 (11%), 16/48 (33%) and 43/49 (88%), respectively. Females received diets containing TWA concentrations of 0, 30.1 and 63.8 mg/kg diet and had incidences of 0/19 (0%), 3/47 (6%) and 34/49 (69%), respectively. Chlordane was not carcinogenic in 50 male and 50 female Osborne-Mendel rats similarly treated with TWA concentrations of chlordane at 120.8-407.0 mg/kg diet (NCI, 1977a). Becker and Sell (1979) found that dietary concentrations of 25 and 50 mg chlordane/kg diet induced primary

hepatocellular carcinoma in unspecified incidences in C57B1/6N mice, a strain that historically and concurrently (200 controls) did not develop spontaneous liver lesions.

Although negative results for mutagenicity of chlordane were obtained in bacterial systems, in mammalian cell cultures for unscheduled DNA synthesis and for the dominant lethal assay in mice (Probst et al., 1981; Gentile et al., 1982; Maslansky and Williams, 1981; Arnold et al., 1977), positive results were obtained in Saccharomyces cerevisiae for mitotic gene conversion in the presence of S-9 and in SV-40 transformed human fibroblast cultures for unscheduled DNA synthesis in the absence of S-9 (Gentile et al., 1982; Ahmed et al., 1977).

Quantification of Carcinogenic Effect. The U.S. EPA (1980b) derived a human carcinogenic potency factor, q_1^* , for chlordane of $1.6075 \text{ (mg/kg/day)}^{-1}$. This derivation was based upon the incidence of hepatic carcinoma in male CD-1 mice of the IRDC (1973a) study as diagnosed by Reuber (Epstein, 1976). This study resulted in greater incidences at lower doses than did the NCI (1977a) bioassay in mice.

The concentration in water corresponding to an increased lifetime risk level of 10^{-4} , 10^{-5} and 10^{-6} for a 70 kg human who consumes 2 l/day, is calculated to be 2.18, 0.218 and 0.0218 $\mu\text{g/l}$, respectively.

Heptachlor and Heptachlor Epoxide

Noncarcinogenic Effects. Unlike the case of technical chlordane, reports of effects in humans swallowing quantifiable amounts of technical heptachlor were not located in the available literature. As in the case of chlordane, exposure levels in reports of blood dyscrasia associated with inhalation and dermal exposure of humans to heptachlor are not quantifiable.

Much of the information on single oral exposures of laboratory animals to heptachlor and heptachlor epoxide is concerned with lethality. Heptachlor is more toxic to laboratory animals than chlordane. Acute oral LD₅₀ values in rats for heptachlor range from 40 mg/kg bw for a commercial formulation (Ben-Dyke et al., 1970) to 162 mg/kg bw for female Sherman rats for technical grade heptachlor (Gaines et al., 1960). Other LD₅₀ values range from 70-100 mg/kg bw in mice and hamsters (Gak et al., 1976). As in the case of chlordane, neonatal rats (i.p. LD₅₀ = 531 mg/kg bw) are far less sensitive to intraperitoneal injections of heptachlor than adult males (i.p. LD₅₀ = 71 mg/kg bw) (Harbison, 1975). For heptachlor epoxide, the acute oral LD₅₀ is ~60 mg/kg bw (Sperling and Ewinike, 1969; Podowski et al., 1979; NAS, 1977). Symptoms of acute intoxication include tumors, convulsions, paralysis, hyperexcitability and irritability (Gaines, 1960; Lehman, 1951; Hrdina et al., 1974).

At a single oral dose of 60 mg heptachlor/kg bw or repeated oral doses of 7 and 12 mg heptachlor/kg bw/day for up to 14 days in rats, significantly increased levels of serum GPT and serum aldolase ($p < 0.05-0.001$) were associated with moderate to severe histological liver damage (Kramp1, 1971). For rats that had been administered these doses for 28 days, however, the serum

levels of these enzymes were not statistically significantly different from controls. The degree of liver damage was less severe than that observed at 7 and 14 days. Rats maintained on diets containing 10 mg heptachlor/kg diet for 1-7 days had evidence of liver damage and altered liver function: increased blood urea, increased blood glucose, decreased liver glycogen content, increased acid and alkaline phosphatase levels when compared with controls (Enan et al., 1982). Liver, heart and spleen weights were also above control weights. Dose-related significant induction of liver microsomal enzymes (aniline hydroxylase, aminopyrine demethylase and hexabarbital oxidase), at dietary levels of heptachlor (>99%) of 2-50 mg/kg diet, was observed in rats (Den Tonkelaar and Van Esch, 1974). The no-effect level was <2 mg/kg diet. However, these investigators did not look for definitive adverse effects. Arnold et al. (1977) reported that a single oral dose of 30 mg/kg bw of a 75% heptachlor epoxide and 25% heptachlor mixture resulted in moderate hypoactivity and 25% mortality in mice.

In dietary studies of 42 days duration, no effects on body weight gain, food consumption or mortality were observed in rats at <80 mg/kg technical heptachlor diet levels or in mice at <40 mg/kg diet levels (NCI, 1977b). Other parameters were not examined. The dietary concentrations are equivalent to doses of 8 mg/kg bw for rats and 12 mg/kg bw for mice, and are quite near the dose levels that resulted in liver damage in rats in the shorter term studies of Krampf (1971). Shain et al. (1977) observed decreased mean weekly body weight gain, reduced food consumption and changes in ventral prostate homeostasis of rats receiving 1.29 mg/kg bw/day of heptachlor. This dose is above the 2 mg/kg diet level for 14 days (i.e., 0.2 mg/kg bw/day) that resulted in enzyme induction in rats (Den Tonkelaar and Van

Esch, 1974), but similar to a dose of 10 mg/kg of diet (i.e., 1.0 mg/kg bw/day) which caused definitive adverse effects in rats (Enan et al., 1982). Kinoshita and Kempf (1970) reported in a meeting abstract that the no-effect level in rats for enzyme induction for heptachlor and heptachlor epoxide was 1 mg/kg diet for 91 days. Although details of this study were unavailable from the abstract, the results support the low-effect level for enzyme induction of 2 mg/kg diet reported by Den Tonkelaar and Van Esch (1974).

Several long-term dietary studies with heptachlor and/or heptachlor epoxide, which were designed as carcinogenicity studies, provide toxicologic data. In the NCI (1977b) bioassay in mice, no histopathological signs of toxicity were observed at TWA concentrations of heptachlor of 6.1-18 mg/kg diet for 80 weeks. However, abdominal distention and increased mortality were observed in the high dose females. The incidences of hepatocellular carcinomas were also significant for high dose males and females. Heptachlor was not carcinogenic in rats, but vaginal bleeding also developed in both groups of treated females (25.7 and 51.3 mg/kg diet).

At dietary levels of 10 mg/kg of heptachlor or heptachlor epoxide in mice, Reuber (1977a) diagnosed hepatic vein thrombosis and cirrhosis of the liver, as well as carcinomas, from slides of the Davis (1965) study. In the IRDC (1973b) study, reviewed by Epstein (1976), a 75% heptachlor epoxide and 25% heptachlor mixture was fed to mice for 18 months. Females and males had dose-related increased mean liver weights and hepatocytomegaly at 1, 5 and 10 mg/kg diet. Jolley et al. (1966) found dose-related increased mortality in rats fed 5-12.5 mg/kg diet levels of a 75% heptachlor and 25% heptachlor epoxide mixture for 2 years. Witherup et al. (1955) found non-neoplastic lesions in rats at dietary levels ≥ 7.0 mg/kg diet for 110 weeks. At < 5.0

mg/kg diet, no lesions were observed. Treated males had dose-related increased liver weights at levels 1.5-10 mg/kg diet. Although this effect is not necessarily adverse, its presence at doses slightly lower than that which caused tumors and the lack of additional histological analysis dictate that this be considered an adverse effect. The 1.5 mg/kg diet level was the lowest concentration of heptachlor tested in any of the chronic studies, and thus represents the LOAEL.

Dose-related liver weight increases, hepatocytomegaly and hepatic cell vacuolization were observed in rats maintained for 108 weeks on diets containing heptachlor epoxide at 0.5-10 mg/kg diet (Witherup et al., 1959). These effects are considered to be adverse. Thus, the 0.5 mg/kg diet level represents a LOAEL for heptachlor epoxide.

Dogs were administered heptachlor epoxide in a diet containing various dose levels for 2 years (U.S. EPA, 1971 IRDC, unpublished report). A level of 1 ppm in diet caused no adverse effect on parameters measured. Dose-related changes in biochemical values related to liver function and microscopic changes in liver were noted at the higher dose levels (3, 5, 7 and 10 ppm in diet).

No evidence of teratogenicity of heptachlor or heptachlor epoxide was available. Mestitzova (1967) reported a marked reduction in litter size, however, in rats that were given heptachlor in the diet for several generations. It was not clear whether the dose was 6 mg/kg bw/day or 6 mg/kg bw over the duration of the experiment.

Quantification of Noncarcinogenic Effects. No quantifiable human data on heptachlor or heptachlor epoxide were available. Thus, it is not possible to determine if humans are more sensitive to these chemicals than laboratory animals. In addition, studies on the effects of heptachlor in rabbits were not available to determine interspecies differences among animals. Heptachlor and chlordane are structurally similar organochlorine pesticides. Technical and commercial preparations of either are often contaminated by the other.

1-Day HA -- A NOEL or a NOAEL could not be defined for heptachlor epoxide in the 1- to 30-day studies. As reviewed above, however, a level of 10 mg/kg diet of heptachlor for 14 days in rats was the lowest concentration that resulted in definite adverse effects (i.e., evidence of liver damage and altered liver function (Enan et al., 1982). Although Den Tonkelaar and Van Esch (1974) reported effects at lower doses (i.e., altered liver enzymes), these investigators did not look for any additional effects and those observed are not necessarily adverse. The former study is preferred over the latter as the basis for a HA, because the former study offers additional details. A daily transformed dose (d) can be derived from the former study by assuming a young rat ingests food equivalent to 10% of its body weight/day. The 1-day HA would be calculated from this transformed dose using a 1000-fold uncertainty factor that represents two 10-fold factors for both intra- and interspecies variability to the toxicity of this chemical in lieu of chemical specific information, and an additional 10-fold factor because the HA is derived from a LOAEL and not a NOAEL. Thus, a 1-day HA is derived as follows:

For a child:

$$1\text{-day HA} = \frac{(1.0 \text{ mg/kg bw}) (10 \text{ kg})}{(1000) (1 \text{ l})} = 0.010 \text{ mg/l}$$

For an adult:

$$1\text{-day HA} = \frac{(1.0 \text{ mg/kg bw}) (70 \text{ kg})}{(1000) (2 \text{ l})} = 0.035 \text{ mg/l}$$

where:

1.0 mg/kg bw = minimal effect level

10 kg = assumed body weight of a child

70 kg = assumed body weight of an adult

1000 = uncertainty factor -- inter- and intraspecies variation and LOAEL

1 l = assumed water consumption per day by a child

2 l = assumed water consumption per day by an adult

10-Day HA -- Few studies of 30-90 days duration that were available defined no- or low-effect levels for heptachlor. In one study that was reported in a meeting abstract (Kinoshita and Kempf, 1970), microsomal enzyme induction occurred in rats in a dose-related way at various dietary levels of heptachlor or heptachlor epoxide administered for 91 days. The NOEL for enzyme induction was 1 mg/kg diet, which is similar to the apparent NOEL for enzyme induction from shorter-term toxicity studies of <2 mg/kg of diet (Den Tonkelaar and Van Esch, 1974).

These results suggest that the rats were either developing a tolerance for heptachlor or responding in a similar fashion as in shorter-term toxicity studies. For these reasons, 10-day HAs for a 10 kg child and

a 70 kg adult should be equal to the recommended 1-day HAs in order to protect from transitory adverse effects on the liver.

For an adult: 10-day HA = 0.035 mg/d

For a child: 10-day HA = 0.010 mg/d

Lifetime AADI -- For chronic exposure the only study that defines NOAELs for heptachlor is that of Witherup et al. (1955). In this study rats exposed to diets containing heptachlor at 1.5-5.0 mg/kg developed a dose-related increase in liver weight. Higher doses caused more severe effects. The lowest dose of 1.5 (0.075 mg/kg bw/day) is considered a LOAEL. Another study by Witherup (1959) also provides a LOAEL for heptachlor epoxide of 0.5 mg/kg (0.025 mg/kg bw/day). Increased liver weight and hepatic cell vacuolization was observed at this dose with higher doses causing more severe effects. Using this study a lifetime AADI for heptachlor and heptachlor epoxide will be calculated using a 1000-fold uncertainty factor that consists of two 10-fold factors to account for both intra- and inter-species variability to the toxicity of these chemicals in lieu of specific information, and an additional 10-fold factor because the ADI is based on a LOAEL and not a NOAEL. These factors are based on previous guidelines (NAS, 1977; Federal Register, 1980).

Heptachlor. The ADI for a 70 kg adult is calculated as follows using 0.075 mg/kg bw/day as the LOAEL in rats (Witherup et al., 1955).

$$ADI = \frac{(0.075 \text{ mg/kg bw/day}) (70 \text{ kg})}{1000} = 0.005 \text{ mg/day}$$

where:

0.075 mg/kg bw/day = minimal effect level in rats

70 kg = assumed body weight of an adult

1000 = uncertainty factor -- intra- and interspecies variation
and LOAEL

Using 0.005 mg/day as the ADI and assuming the 70 kg adult consumes
2 l of drinking water per day, the AADI is calculated as follows:

$$\frac{0.005 \text{ mg/day}}{2 \text{ l/day}} = 0.0025 \text{ mg/l}$$

Assuming 20% relative source contribution from drinking water, the AADI
would be:

$$\frac{(0.005 \text{ mg/day}) (0.20)}{2 \text{ l/day}} = 0.0005 \text{ mg/l}$$

Heptachlor Epoxide. The ADI for a 70 kg adult is calculated as
follows using 0.025 mg/kg bw/day as the NOAEL in dogs (U.S. EPA, 1971 - IRDC
unpublished report).

$$\text{ADI} = \frac{(0.025 \text{ mg/kg bw/day}) (70 \text{ kg})}{1000} = 0.00175 \text{ mg/day} = 0.002 \text{ mg/day}$$

where:

0.025 mg/kg bw/day = no-observed-adverse-effect level in dogs

70 kg = assumed body weight of an adult

1000 = uncertainty factor -- intra- and interspecies variation
and certain limited measurements

Using 0.002 mg/day as the ADI for heptachlor epoxide and assuming the 70 kg adult consumes 2 l of drinking water per day, the AADI is calculated as follows:

$$\frac{(0.002 \text{ mg/day})}{2 \text{ l/day}} = 0.001 \text{ mg/l}$$

Assuming 20% relative source contribution from drinking water, the AADI would be:

$$\frac{(0.002 \text{ mg/day}) (0.20)}{2 \text{ l}} = 0.00002 \text{ mg/l}$$

A summary of the data used to calculate the recommended 1-day and 10-day HAs and the lifetime AADI for heptachlor/heptachlor epoxide is provided in Table VIII-2.

Carcinogenic Effects. Since heptachlor is a contaminant or component of chlordane formulations, the reported cases of neuroblastoma (Infante et al., 1978) and other tumor types associated with chlordane exposure (Wang and MacMahon, 1979a,b; Ditraglia et al., 1981) may also apply to heptachlor exposure.

Heptachlor and heptachlor epoxide have been studied for carcinogenicity in mice and rats. Statistically significant increased incidences of hepatic carcinoma were determined by Reuber and by Williams upon re-analysis of histologic slides from the Witherup et al. (1959) study of heptachlor epoxide at 0, 0.5, 2.5, 5.0, 7.5 and 10.0 mg/kg diet in CFN rats for 108 weeks.

TABLE VIII-2
Summary of Data Used to Derive HAs and AADIs for Heptachlor/Heptachlor Epoxide

Criteria	Animal Dose	Duration	Effect	Value of HA or AADI		Reference
				Child	Adult	
1-day HA	1.0 mg/kg bw/day	14 days	Lowest level for evidence of liver damage and altered liver function	10 µg/l (10 µg/day or 1.0 µg/kg bw)	35 µg/l (70 µg/day or 1.0 µg/kg bw)	Enan et al, 1962
10-day HA			As above for the 1-day HA			
Lifetime AADI for heptachlor	0.075 mg/kg/day	110 weeks	Lowest level for increased liver weight	NA	2.5 µg/l (100% RSC)* or 0.5 µg/l (20% RSC)*	Witherup et al., 1955
Lifetime AADI for heptachlor epoxide	0.025 mg/kg bw/day	2 years	NOAEL	NA	1.0 µg/l (100% RSC) or 0.2 µg/l (20% RSC)	U.S. EPA, 1971 -IRDC unpublished report

*RSC: Percent relative source contribution being from water

NA - Not applicable

The incidences of hepatic carcinomas as determined by Reuber were as follows: for males, 1/24 (4%), 1/22 (5%), 0/19 (0%), 1/23 (4%), 1/25 (4%) and 4/22 (18%), respectively; for females, 0/17 (0%), 3/22 (14%), 3/18 (17%), 7/22 (32%), 3/21 (14%) and 5/19 (26%), respectively. The incidences were statistically significantly different from controls for female rats at the 5.0 and 10.0 mg/kg diet concentration (Epstein, 1976). Histological re-examination of the slides of the Davis (1965) study by Reuber (and confirmed by three other pathologists) resulted in incidences of hepatocellular carcinoma in C3H mice of 22/73 (30%) control males, 2/54 (4%) control females, 64/87 (74%) heptachlor males, 57/78 (73%) heptachlor females, 73/79 (92%) heptachlor epoxide males and 77/81 (95%) heptachlor epoxide females. The concentration of heptachlor or heptachlor epoxide was 10 mg/kg diet (Epstein, 1976). Histologic re-evaluation of slides of the IRDC (1973b) study, on a 75% heptachlor epoxide, 25% heptachlor mixture in CD-1 mice, by Reuber (and supported by five other pathologists) resulted in incidences of hepatocellular carcinoma of 0/62 (0%) male controls, 2/61 (3%) males at 1.0 mg/kg diet, 18/68 (26%) males at 5.0 mg/kg diet, 52/80 (65%) males at 10.0 mg/kg diet, 6/76 (8%) control females, 1/70 (1%) females at 1.0 mg/kg diet, 6/65 (9%) females at 5.0 mg/kg diet and 30/57 (52%) females at 10.0 mg/kg diet.

In an NCI (1977b) bioassay, male B6C3F₁ mice received TWA dietary concentrations of 0, 6.1 and 13.8 mg heptachlor/kg diet and had incidences of hepatocellular carcinoma of 5/19 (26%), 11/46 (24%) and 34/47 (72%), respectively. Females received diets containing TWA concentrations of 0, 9.0 and 18.0 mg/kg diet and had incidences of carcinoma of 2/10 (20%), 3/47 (6%) and 30/42 (71%), respectively. The incidences in high-dose males was

statistically significant. A highly significant dose-related trend was observed between high- and low-dose females. High-dose females had significantly greater incidences than controls. Heptachlor was not carcinogenic in 50 male and 50 female Osborne-Mendel rats similarly treated with TMA concentrations of heptachlor of 25.7-77.9 mg/kg diet (NCI, 1977b).

Heptachlor was negative for mutagenicity in bacterial systems (Moriya et al., 1983; Probst et al., 1981; Gentile et al., 1982; Shirasu et al., 1976), in mitotic gene conversion (Gentile et al., 1982), for the recessive lethal assay in fruit flies (Benes and Sram, 1969), for unscheduled DNA synthesis in rat, mouse and hamster primary hepatocyte cultures (Probst et al., 1981; Maslansky and Williams, 1981) and for the dominant lethal assay in mice (Arnold et al., 1977). Positive results were obtained in the dominant lethal assay in rats (Cerey et al., 1973) and for unscheduled DNA synthesis in SV-40 transformed human fibroblasts with S-9 activation (Ahmed et al., 1977).

Heptachlor epoxide was negative in bacterial systems (Moriya et al., 1983; Marshall et al., 1976), in the recessive lethal assay in fruit flies (Benes and Sram, 1969) and in the dominant lethal assay in mice (Arnold et al., 1977). Heptachlor epoxide was positive for unscheduled DNA synthesis in human fibroblasts in the presence of S-9 (Ahmed et al., 1977).

Quantification of Carcinogenic Effects. The U.S. EPA (1980a) derived a human carcinogenic potency factor, q_1^* , for heptachlor of $3.37 \text{ (mg/kg/day)}^{-1}$. This derivation was based upon the incidence of hepatocellular carcinoma in male B6C3F₁ mice of the NCI (1977b) bioassay.

According to the U.S. EPA (1980a), the concentration of heptachlor in water corresponding to an increased lifetime risk level of 10^{-4} for a 70 kg human who consumes 2 L/day is calculated, for example, from the following equation:

$$\text{concentration in water} = 10^{-4} \times 70 \text{ kg} \div q_1^* (\text{mg/kg/day})^{-1} \times 2 \text{ L}$$

Thus, using the q_1^* of $3.37 (\text{mg/kg/day})^{-1}$, the concentrations of heptachlor in water corresponding to increased lifetime risks of 10^{-4} , 10^{-5} and 10^{-6} are 1.04, 0.104 and 0.0104 $\mu\text{g/L}$, respectively. The NAS (1977) determined 0.119 $\mu\text{g/L}$ for heptachlor as the water concentration corresponding to an increased lifetime risk of cancer of 10^{-5} .

The U.S. EPA (1980a) did not report a q_1^* for heptachlor epoxide. In two unpublished studies reviewed by Epstein (1976), statistically significant increased incidences of liver carcinoma were reported on the basis of Dr. Reuber's rediagnoses, which were supported by other pathologists. In Davis (1965), male and female C3H mice received 0 and 10 mg/kg diet of heptachlor epoxide for 728 days. Assuming a mouse consumes an amount of food equal to 13% of its body weight, $10 \text{ mg/kg diet} \times 0.13 = 1.3 \text{ mg/kg bw/day}$. Based on the carcinoma response of male C3H mice (73/79 treated versus 22/73 control), and using the linearized multistage model developed by Crump and adopted by U.S. EPA (1980c), a carcinogenic potency factor (q_1^*) of $30.24 (\text{mg/kg/day})^{-1}$ can be derived for humans. Using the carcinoma response of female mice (77/81 (95%) treated versus 2/54 (4%) control), a q_1^* of $39.92 (\text{mg/kg/day})^{-1}$ is derived. In Witherup et al. (1959), 25 male and 25 female CFM rats were maintained on diets containing

heptachlor epoxide at concentrations of 0, 0.5, 2.5, 5.0, 7.5 and 10.0 mg/kg diet for 756 days. Assuming that a rat consumes an amount of food equal to 5% of its body weight, the corresponding doses are 0, 0.025, 0.125, 0.25, 0.375 and 0.5 mg/kg bw/day, respectively. Based upon the liver carcinoma response in female rats (0/17 (0%), 3/22 (14%), 3/18 (17%), 7/22 (31%), 3/21 (14%), 5/19 (26%), respectively), and using the linearized multistage model (U.S. EPA, 1980c), a q_1^* for humans of $57.86 \text{ (mg/kg/day)}^{-1}$ is derived. Inspection of tumor incidence with respect to dose reveals that the dose-response effect is nonlinear. A chi-square test of fit was performed for all doses according to the method recommended by U.S. EPA (1980c), with the result that the fit is acceptable. Since the q_1^* , $57.86 \text{ (mg/kg/day)}^{-1}$, derived from the data in female rats (Witherup et al., 1959) is a more conservative value than that derived from either the male or female mouse data of Davis (1965), and since there is no apparent greater merit of one study over the other, the value of $q_1^* = 57.86 \text{ (mg/kg/day)}^{-1}$ is used to calculate the concentrations of heptachlor epoxide in water corresponding to increased lifetime risks of 10^{-4} , 10^{-5} and 10^{-6} for a 70-kg human who consumes 2 L/day. The corresponding concentrations are 60.49, 6.049 and 0.6049 ng/L, respectively. However, it should be noted that the NAS (1977) has stated that "Before limits for...heptachlor epoxide in drinking water can be established, more toxicological data must be gathered and evaluated." NAS (1977) categorizes heptachlor epoxide as a suspected animal carcinogen but notes that there are "insufficient data to permit a statistical extrapolation of risk." In addition, the position of the IARC (1977) is that "There is limited evidence that heptachlor epoxide is carcinogenic in experimental animals."

A comparison of values for q_1^* derived from the various studies discussed above is presented in Table VIII-3. For convenience, the concentrations corresponding to increased lifetime risk of 10^{-3} are included where appropriate. Complete data for derivation of the q_1^* s are presented in Appendices I and II.

Special Considerations

High Risk Subpopulations. High risk subpopulations have not been identified in the available literature. Cases of blood dyscrasia have been reported in association with human exposure to chlordane and heptachlor, as well as with other pesticides (Infante et al., 1978; Furie and Trubowitz, 1976; Klemmer et al., 1977). Infante et al. (1978) suggested that an idiosyncratic mechanism for susceptible, but not readily identifiable, individuals may be involved.

Groups that may be or might have been more affected by high exposure rather than by intrinsic susceptibility include exterminators, agricultural applicators, lawn sprayers and workers involved in the manufacture of pesticides. High exposure may also result in persons who live in areas of high pesticide use. Breast-fed infants may be exposed to high levels, since heptachlor epoxide, a major metabolite of heptachlor, has been detected in numerous samples of human milk (Kroger, 1972; Ritcey et al., 1972; Savage et al., 1973; Bakken and Seip, 1976; Polishuk et al., 1977b; Jonsson et al., 1977; Strassman and Kutz, 1977). Oxychlordane, a metabolite of chlordane, was also detected in human milk (Strassman and Kutz, 1977). Developing fetuses may be exposed in utero. The transplacental transfer of these

TABLE VIII-3

Summary of q_1^* Determination and Concentrations for Increased Lifetime Risk of 10^{-5}

Compound	Species and Sex	q_1^* (mg/kg/day) ⁻¹	Concentration for Increased [†] Lifetime Risk of 10^{-5}	Reference
Chlordane	male CD-1 mice	1.6075	0.218 μ g/ μ	IRDC, 1973a; Epstein, 1976
Heptachlor	male B6C3F ₁ mice	3.37	0.104 μ g/ μ	NCI, 1977b
Heptachlor epoxide	male C3H mice	30.24	NA	Davis, 1965; Reuber, 1977a; Epstein, 1976
	female C3H mice	39.92	NA	Davis, 1965; Reuber, 1977a; Epstein, 1976
	female CFN rats	57.86	6.049 ng/ μ	Witherup et al., 1959; Epstein, 1976

[†]Concentrations associated with other lifetime excess cancer risks can be estimated by division or multiplication of the 10^{-5} level by 10. For example the concentration associated with a lifetime excess cancer risk of 10^{-6} is 0.0218 μ g/ μ (i.e., 0.218 μ g/ μ + 10 = 0.0218 μ g/ μ).

NA = Not applicable

chemicals is well documented (Curley et al., 1969; Wasserman et al., 1972, 1974; Zvon et al., 1969; Selby et al., 1969; Polishuk et al., 1977a).

Multiple Pollutant Exposures. Pretreatment of adult male rats with turpentine potentiated the toxicity of heptachlor, reducing the oral LD₅₀ from 112 to 70 mg/kg bw (Sperling and Ewinike, 1969). The toxicity of heptachlor epoxide was unaffected. Pretreatment of rats with chlordane resulted in enhancement of hepatocellular necrosis induced by CCl₄ (Stenger et al., 1975).

Several cases of mixed exposure with other pesticides were reported to be associated with blood dyscrasias in humans. These include hemolytic anemia associated with exposure to chlordane, heptachlor, dieldrin and toxaphene (Muirhead et al., 1959); and aplastic anemias, pancytopenia, thrombocytopenia, leukopenia and agranulocytosis associated with chlordane and heptachlor, as well as with other pesticides such as lindane and chlorophenothane (Loge, 1965), Dicamba, Diazinon and 2,4-D (Infante et al., 1978).

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APPENDIX I

Cancer Data Sheet for Derivation of q_1^*

Compound: Heptachlor epoxide

Reference: Reuber's re-analysis of Davis (1965)
Reviewed by Epstein (1976)

Species, Strain, Sex: Mouse, C3H, male and female

Body Weight: 0.035 kg (assumed)

Length of Exposure (t_e) = 728 days

Length of Experiment (L_e) = 728 days

Lifespan of Animal (L) = 728 days

Tumor Site and Type: liver, carcinoma

Route, Vehicle: oral, diet

Experimental Doses or Exposures		Transformed Dose (mg/kg/day)	Incidence No. Responding/No. Tested (or examined)
Male	0	0	22/73
	10 mg/kg diet	1.3	73/79
Female	0	0	2/54
	10 mg/kg diet	1.3	77/81

Unadjusted q_1^* from study = 2.2796 (male); 3.0063 (female) (mg/kg/day)⁻¹

Human q_1^* = 30.24 (male); 39.93 (female) (mg/kg/day)⁻¹

APPENDIX II

Cancer Data Sheet for Derivation of q_1^*

Compound: Heptachlor epoxide

Reference: Reuber's re-analysis of Witherup et al. (1959)
Reviewed by Epstein (1976)

Species, Strain, Sex: Rat, CFN, female

Body Weight: 0.35 kg (assumed)

Length of Exposure (le) = 756 days

Length of Experiment (Le) = 756 days

Lifespan of Animal (L) = 756 days

Tumor Site and Type: liver, carcinoma

Route, Vehicle: oral, diet

Experimental Doses or Exposures (mg/kg diet)	Transformed Dose (mg/kg/day)	Incidence No. Responding/No. Tested (or examined)
0	0	0/17
0.5	0.025	3/22
2.5	0.125	3/18
5.0	0.250	7/22
7.5	0.375	3/21
10.0	0.50	5/19

Unadjusted q_1^* from study = $9.8942 \text{ (mg/kg/day)}^{-1}$

Human q_1^* = $57.86 \text{ (mg/kg/day)}^{-1}$